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PARASITOLOGY

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PARASITOLOGY

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ANTONY VAN LEEUWENHOEK

1632—1725

ON TWO NEW TAPEWORMS FROM THE OSTRICH, WITH
A KEY TO THE SPECIES OF *DAVAINEA*.

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(*From the Research Department in Agricultural Zoology,
University of Birmingham.*)

(With Plate I and 2 Text-figs.)

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THE material which forms the basis of the following account consists of two tubes of tapeworms collected from ostriches at the Veterinary Pathological Laboratory, Nairobi, British East Africa. One tube contained approximately 12 scolices and the anterior portions of the strobilae attached to them, together with several fragments of the more posterior portions: these are described under the name *Davainea beddardi* n. sp. The other tube contained one large fragment of a *Davainea* and several smaller pieces but no scolices: these are described under the heading *Davainea* sp. For this material I am indebted to Professor G. H. F. Nuttall, F.R.S. to whom I wish here to express my thanks. I have also to acknowledge my indebtedness to the Librarian of the Royal College of Veterinary Surgeons for allowing me to consult literature unobtainable elsewhere.

***Davainea beddardi* n.sp.**

The length of the largest fragment was 6·4 mm. and the greatest breadth observed 3 mm.

The scolex (Plate I, fig. 2) is 1·3 mm. diameter, possessing four unarmed suckers and a rostellum bearing a double row of approximately 130 hooks, each 0·085–0·088 mm. long and of the typical *Davainea* shape, the two rows of

hooks exhibiting a rather irregular alternation of large and small forms. The rostellum itself consists of a short broad central portion, 0.48 mm. diameter, bearing the hooks, surrounded by a band of parenchymatous tissue, 0.81 mm. diameter, in shape like a pneumatic tyre, the whole bearing a remarkable resemblance to the scolex (Plate I, fig. 1) of *Davainea brotogeris* Meggitt, 1915. This appearance has been figured by Zilluf (1912, 17) for a form he calls *Davainea struthionis* but which is instead probably identical with *D. beddardi*. With the exception of the armed collar posterior to the rostellum, figured by Dujardin (1845, Plate 9, fig. LI) and mentioned by Stossich (1895) and Blanchard (1891 a, 435) for *D. frontina* (Duj.), in no other species of *Cotugnia* or *Davainea* has this modification of the scolex been mentioned, and absence of comment in the case of so noticeable a structure must be held to imply the absence of the structure itself. It is impossible for this appearance to have been caused by the method of fixation or for it to be a temporary character in any way. It was characteristic of each of the 12 scolices of *D. beddardi* and all those of *C. brotogeris*, although the two forms were fixed by different reagents. The close similarity of the two scolices is interesting in view of the fact that one genus has a double and the other only a single set of genital organs in each proglottis, and forms yet another comment, if one were needed, upon the inadvisability of distinguishing species by the size and external appearance of the scolex alone unaccompanied by any internal morphological difference. A neck is absent. All proglottides seen were broader than long: in those containing mature eggs the length approximated to the breadth.

The longitudinal musculature (Plate I, fig. 3) is arranged in two layers, a stronger internal one of numerous bundles of small fibres and a weaker external one, often absent, of isolated fibres. Separating these two is a thin layer of transverse muscles, while a second band exists internally to the inner longitudinal muscle layer. In the extreme anterior portion of the strobilus (Plate I, fig. 4), the two longitudinal muscle layers merge into one, the separating transverse muscles disappearing. The genital pore is unilateral opening at the posterior two-thirds of the proglottis margin. There is a deep narrow genital cloaca at the bottom of which open the genital ducts. Both male and female ducts pass dorsal to the longitudinal excretory vessels. The cirrus-sac is small, not extending beyond the longitudinal nerve-cord and, as far as could be ascertained in the state of preservation of the material, had a very feeble muscular structure. The cirrus is unarmed and that portion of the vas deferens inside the cirrus-sac is coiled several times in the form of a very twisted piece of rope. The coils of the vas deferens stretch from the inner end of the pouch half-way across the proglottis, then break up into the vasa efferentia. Neither vesicula seminalis nor sacculus accessorius could be observed. The testes are small, approximately 100 in number, and fill the whole of the dorsal surface of the proglottis, extending laterally to the longitudinal excretory vessels. Proglottides containing the sexually mature female organs were absent. From the immature segments found, it could be

ascertained that a small spindle-shaped receptaculum seminis is present and that the female glands are posterior and ventral.

The onchospheres, in groups of 5–8, are enclosed in capsules which fill the whole field, extending laterally beyond the longitudinal excretory vessels but not beyond the nerve.

The only species of tapeworm so far recorded from the ostrich belong to the genus *Davainea*. The first mention of any Cestode parasites from this host was by Houttuyn (1773) and Rudolphi (1810 and 1819) who listed under the name of *Taenia struthionis* a form from *Struthio camelus*. Their records were unaccompanied by any description whatsoever, and it was not until the appearance of Parona's paper (1885) that the species mentioned by the two previous authors was given characters which adequately distinguished it. A few years later it was placed by Fuhrmann (1896, 128) in the new genus *Davainea*. In 1893, von Linstow described from *Struthio molybdophanos* forms which he regarded as belonging to this species, but at the same time found certain discrepancies between his own observations and Parona's account. From *Struthio australis* L. (= *S. australis* Gurn.?) Hungerbühler (1910, 511) later described under the name *Davainea struthionis* (Houtt.) cestodes which he believed identical with both Parona's and von Linstow's specimens but which differed from those of the latter in having a rostellum armed with numerous rows of fine thorn-shaped hooks, 0.005 mm. long, in addition to the usual double circle of rostellar hooks. No further investigations, beyond those of Zilluf (1912) on the musculature of the scolex, were made until 1915 when Beddard showed, while describing the anatomy of some *Davaineas* from *Struthio masaiicus*, that the descriptions of von Linstow and Parona applied to separate species, thus accounting for the discrepancies mentioned above. His own specimens, he concluded, might or might not be identical with those found by Parona, but were certainly different from those found by von Linstow. Speaking of his own and Parona's forms, he states (p. 601), "If we can trust as differential characters the diameter of the proglottides and the size of the scolex then the two forms are different. There are no other data that seem to permit of a more definite expression of opinion," and on these grounds does not name his specimens. At this time there are thus two well-defined species of tapeworms parasitic in *Struthio*, one from *S. molybdophanos* described by von Linstow, and the other described by Parona and Beddard from *S. camelus* and *S. masaiicus* respectively. Hungerbühler's specimens from *S. australis* agree best with those described by von Linstow, the scolex and cirrus-sac being of approximately the same size, while the characters in which they differ, the presence of a rostellum and its additional armature, are such as would not be apparent in poorly preserved specimens and so may possibly have been overlooked by von Linstow.

From the first of these two species the form found at Nairobi differs in the presence of calcareous corpuscles and of a rostellum, in the absence of pigment in the scolex, in the shape of the hooks and in the length of the

Tapeworms from Ostrich

cirrus-sac: from the second it is distinguished by having the eggs enclosed in groups in capsules. Neither does it agree with any of the species of *Davainea* previously described from other hosts. For its reception therefore, there must be formed a new species, for which I suggest the name *Davainea beddardi* n. sp. after Dr Beddard whose paper has largely dissipated the confusion formerly associated with Parona's *D. struthionis*. The following table gives the characters in which the three species differ one from the other.

	<i>Davainea struthionis</i> Parona	<i>Davainea struthionis</i> v. Linstow	<i>Davainea beddardi</i> n. sp.
Scolex	2 mm. diam. quadrate Parona 1.2 mm. diam. Beddard	1.125–1.18 mm. diam.	1.3 mm. diam. glo- bular
Rostellum	present	absent (?)	present
Rostellar hooks (for shape see Text-fig. 1)	130, 0.02–0.03 mm. long	164, 0.075–0.084 mm. long	130, 0.085–0.088 mm. long
Suckers	non-pigmented	pigmented	non-pigmented
Calcareous corpuscles	present in scolex	absent from scolex	present in scolex
Cirrus-sac	reaches nerve cord	reaches ventral longi- tudinal excretory vessel	reaches nerve cord
Genital pore	in anterior part of pro- glottis margin		in posterior part of proglottis margin
Eggs	singly in capsules	capsules contain 8–12	capsules contain 5–8
Host	<i>S. camelus</i> <i>S. masaicus</i>	<i>S. molybdophanos</i>	<i>S. masaicus</i>

In order that confusion may not be caused by the use of the same name for two separate species, I venture to separate von Linstow's form from Parona's under the name of *Davainea linstowi* and to give a diagnosis of the specific characters of each of the three species of tapeworms so far recorded from the ostrich. These characters include those mentioned by Dr Beddard and others taken from the original drawings and descriptions.

D. struthionis (Parona, 1885).

Length 270 mm., greatest breadth of proglottides 8.5–9 mm. Scolex 2 mm., diam., quadrate, with rostellum bearing a double circle of hooks 0.02–0.03 mm. long, the shorter and larger hooks alternating. Suckers unarmed. Genital pores unilateral, anterior. Cirrus-sac reaching to nerve-cord. Ova 0.03 mm. long \times 0.02 mm. broad, singly in egg-capsules extending laterally to ventral longitudinal canal.

HOST.	<i>Struthio camelus</i> L., <i>S. masaicus</i> Neu.
LITERATURE.	Parona, 1885 (<i>Taenia struthionis</i>). Beddard, 1915 (<i>Davainea struthionis</i>). Blanchard, 1891 (<i>Davainea struthionis</i>).

D. linstowi n. sp.

Length 160–620 mm., greatest breadth of proglottides 4.43 mm. Scolex 1.18 mm. diam., armed with 164 hooks, 0.084–0.075 mm. long, in two rows, the

longer and shorter forms alternating. Rostellum absent (?). Suckers unarmed, pigmented. Calcareous corpuscles absent anteriorly. Genital pores unilateral, posterior. Cirrus-sac reaches ventral excretory canal. Ova 0.052 mm. diam., 8-12 in each capsule.

HOST.	<i>Struthio molybdophanos</i> Reichen, <i>S. australis</i> Gurn.
LITERATURE.	von Linstow, 1893 (<i>Taenia struthionis</i>). Blanchard, 1899a (<i>Davainea struthionis</i>). Hungerbühler, 1910 (<i>Davainea struthionis</i>).

D. beddardi, n. sp.

Length ? , greatest breadth of proglottides 3 mm. Scolex 1.3 mm. diam., globular, with rostellum armed with 130 hooks 0.085-0.088 mm. long, in two rows. Suckers unarmed. Calcareous corpuscles numerous in scolex. Genital pores unilateral, posterior. Cirrus-sac reaching to nerve-cord. Ova in groups of 5-8 in capsules extending laterally to ventral longitudinal canal.

HOST.	<i>S. masaicus</i> .
LITERATURE.	Present paper. Zilluf, 1912 (<i>Davainea struthionis</i>).

Davainea sp.

The scolex and anterior portion of the strobilus were not seen. The inner longitudinal musculature (Plate I, fig. 5) consists of two layers, an inner and an outer, separated by a band of very weak transverse muscles. The inner longitudinal muscles form a single row of bundles, each composed of many (25-40) separate fibres; in some cases the bundles are entirely distinct one from the other, in others so crushed together that they appear as one continuous layer. Normally there is a certain amount of intermingling but the limits of the individual bundles can usually be ascertained. This layer is bounded internally by a strong band of transverse muscles. The outer longitudinal muscle layer is much weaker and the bundles more scattered than is the case with the inner. Its inner limit is formed by scattered bundles invariably distinct one from the other and consisting of but few fibres (3-6). From this, smaller bundles and isolated muscle strands are strewn through the cortex to the limits of the cuticula, the stronger muscle groups being the more internal and the fibres becoming successively weaker the more they approach the cuticula. In addition to these layers, a plate of dorso-ventral fibres separates each proglottis from its neighbours.

The excretory system consists of two longitudinal vessels only, united posteriorly in each proglottis by a commissure. Dorsal longitudinal vessels could not be seen.

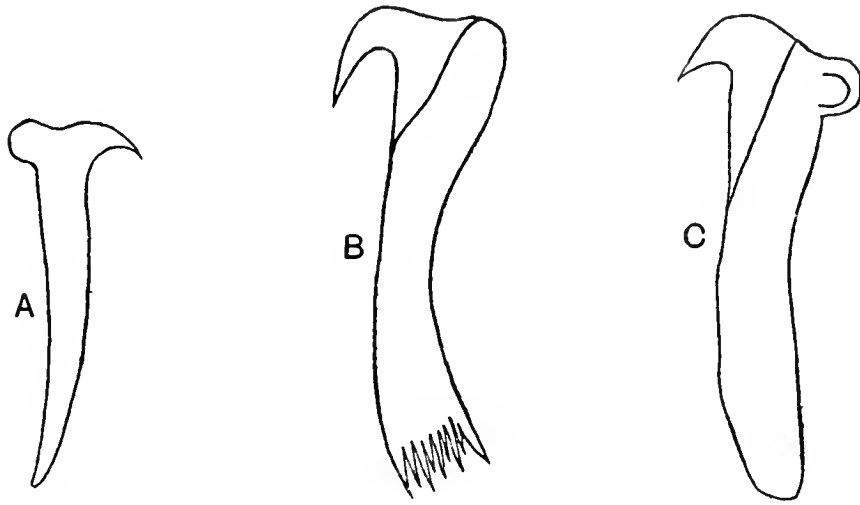
The genital pore (Plate I, fig. 7) is unilateral, at the posterior three-quarters of the proglottis margin. The genital cloaca is comparatively shallow and leads diagonally and anteriorly into the proglottis. The cirrus-sac is long and slender, only reaching to the nerve-cord, and of very feeble muscular

structure. It runs diagonally nearly to the anterior border of the proglottis and at approximately two-thirds of its length makes a right-angled turn towards the dorsal surface. The cirrus is short, uncoiled, and unarmed, and opens into the vas deferens while still only half-way along the cirrus-sac. After a few coils which fill up the remainder of the cirrus-sac, the vas deferens emerges from the inner extremity and extends as a coiled duct along the antero-dorsal surface of the proglottis to a point half-way between the excretory vessels, there splitting up into the vasa efferentia. The testes are numerous, approximately 200 in number and of normal size. They fill the whole dorsal surface of the proglottis, extending laterally to the longitudinal excretory vessels, but with fewer on the poral side than on the aporal. In the centre of the proglottis, the layer is two or sometimes three testes deep, towards the lateral margins only one deep.

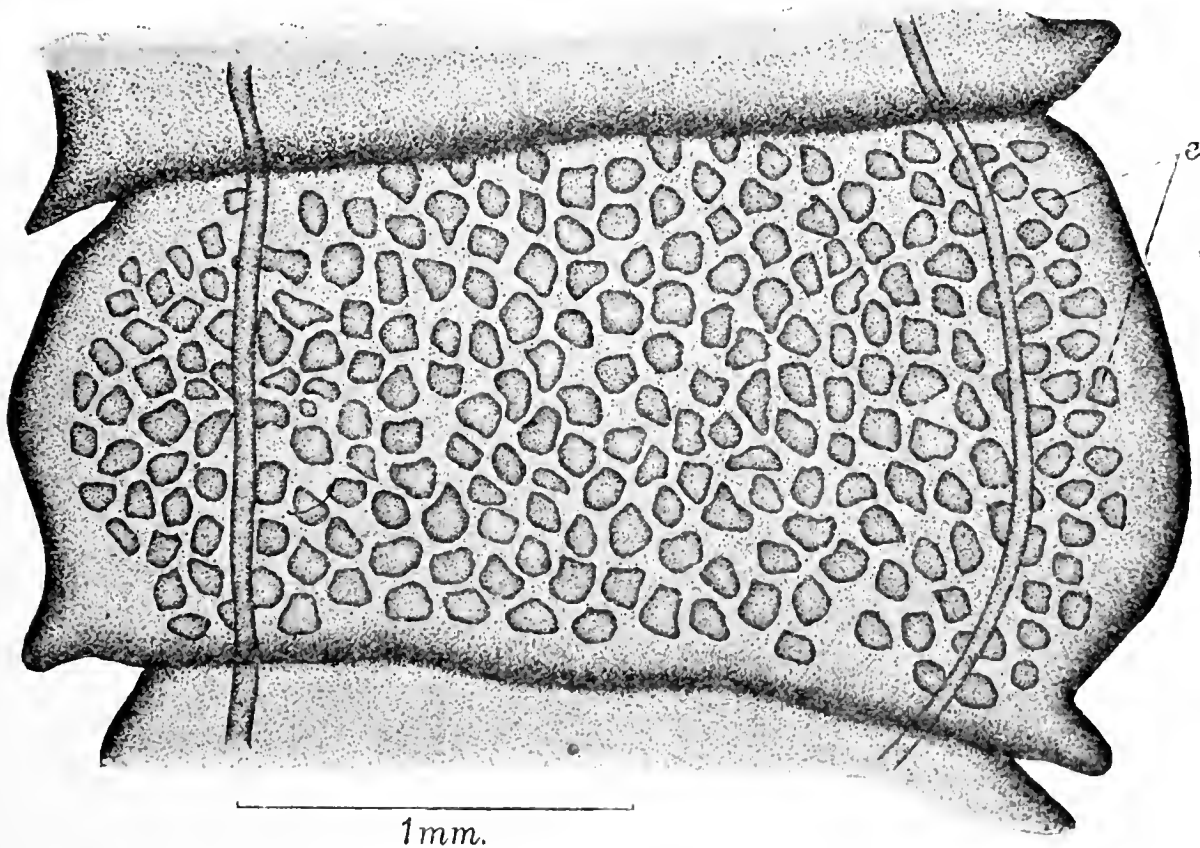
The vagina is devoid of a sphincter muscle. It runs posteriorly to the cirrus-sac and then proceeds transversely across the proglottis, midway between the dorsal and ventral surfaces, passing the excretory vessels. At one-third of the proglottis breadth it opens into the receptaculum seminis. This latter is a narrow thin-walled tube gradually decreasing in diameter as it approaches the centre of the proglottis. It is always choked with spermatozoa. The ovary is a comparatively small but very much lobed gland, lying on the ventral surface of the proglottis with the lobes directed towards the dorsal surface; its ventral aspect is flat. It lies ventral and slightly posterior to the inner end of the receptaculum seminis. Posteriorly to it and of a slightly smaller size is the yolk-gland. This is bilobed, with the two lobes united by a narrow bridge from which springs the vitelline duct. It is more compact than the ovary. The shell-gland is in the form of a sphere of cells united at their inner extremities and grouped round the oviduct, and lies upon the dorsal surface half-way between the yolk-gland and ovary. Inside it the oviduct coils slightly before receiving the vitelline duct. A definite but ephemeral uterus is developed, lying transversely across the proglottis, anteriorly to the female glands and inserting itself among the testes on the dorsal surface. It never passes beyond the stage of a small tube, slightly enlarged where the oviduct joins it, and quickly breaks up into egg-capsules, approximately 140 in number, which fill the whole proglottis, extending laterally beyond the longitudinal excretory vessels (Text-fig. 2). Each capsule usually contains six eggs, and is polygonal in shape owing to compression by its neighbours. The interior (Plate I, fig. 6) is filled with a fibrous mass in which the eggs are embedded and which is further partially sub-divided by a dark homogeneous substance similar in appearance to the capsule wall. There is no tendency to isolate separate eggs in the capsule, the partial compartment formed sometimes containing several eggs and sometimes being empty. Also no case could be seen in which the eggs appear each in a separate capsule or scattered through the parenchyma as in other species of *Davainea*. The capsule is far too definite

for it to be a temporary structure or for it to be split up into secondary capsules as in *D. polycalceola* Jan. A similar case has been described by Mola (1907 *a*, p. 578) for *D. hertwigi*: “La vasta cavità uterina è suddivisa imperfettamente in numerosa e piccole cellette, da tessuto parenchimatoso, nelle quali si trovano le uove in numero di 1–3 con le larve esacante.”

In comparing this species of *Davainea* with others, the one it most resembles is the previously described *D. beddardi*. In both species the unilateral



Text-fig. 1. Rostellar hooks of Cestodes from the ostrich.
a, after Parona; b, after von Linstow; c, author's figure.



Text-fig. 2. Proglottis of *Davainea* sp., filled with egg-capsules (e).

genital pores are situated posteriorly along the proglottis margin, the capsules each contain approximately the same number of eggs and extend laterally beyond the longitudinal excretory vessel, and the relative length of the cirrus-sac (only reaching the nerve-cord) is the same. On the other hand they differ markedly in outward appearance and width of the proglottides, in the number of testes—*D. sp.* having approximately twice as many as *D. beddardi*—and in the cirrus-sac being further posterior in the former than in the latter.

A dorsal longitudinal vessel was observed in *D. beddardi*, but could not be found in the other form. The musculature in the former is also distinctly weaker and more diffuse than in the latter, while at the same time not extending so far into the cortex. These differences appear to indicate the existence of two separate species. Considering however that the scolex of the one form is unknown, that the definition of the other was drawn up from fragments, and that both came from the same species of host, the differences above enumerated are certainly not sufficient to justify the creation of two separate species for the two forms. It therefore seems best to leave this form unnamed until its scolex or more complete specimens of *D. beddardi* be found.

The Genus *Davainea*.

The genus *Davainea* was erected by Blanchard and Railliet in 1891 for the reception of Cestodes characterised by the possession of T-shaped rostellar hooks, armed suckers, and the absence of a persistent uterus. In the same year Blanchard (1891 *a*, p. 429) defined it as follows: "Vers de taille petite ou moyenne. Tête arrondie, surmontée d'un rostre ou creusée d'un infundibulum, mais armée dans l'un et l'autre cas d'une double couronne de crochets nombreux, petits et d'une forme spéciale. Ventouses arrondies, entourées de plusieurs rangées de petits crochets ou spicules, caducs ou persistant pendant toute la vie. Pores génitaux unilatéraux ou irrégulièrement alternes. Dans l'anneau mûr, d'ordinaire beaucoup de corpuscules arrondis, séparés les uns des autres et formés d'un grand nombre d'œufs conglomérés, dépourvus d'appareil piriforme; parfois aussi, œufs isolés, épars dans le parenchyme de l'anneau. Développement inconnu; on suppose que la larve de certaines espèces vit dans la cavité générale des Myriapodes et des Mollusques terrestres. Le Ver adulte est parasite de l'intestine grêle des Oiseau (Coureurs, Gallinacés, Pigeons) et même de l'Homme." The number of species to be assigned to it was estimated by that author as 14, or possibly 16. Fuhrmann (1896, p. 127) later increased that number to 23—two of which however [*D. tauricollis* (Chap.) = *Chapmania tauricollis* (Chap.) and *D. (?) clavulus* (v. Lins.) = *Bi-uterina clavulus* (v. Lins.)] are now placed in other genera—but "Von diesen 23 Vertretern des Genus *Davainea* sind nur 10 anatomische genauer untersucht." Since that date our knowledge of the genus has been increased greatly, largely owing to the work of the last named author, until in 1908 the number of species of *Davainea* from birds amounted to 59, collected from 74 hosts (Fuhrmann, 1908). To this number must be added 31 others described in the last ten years which, together with the 16 species from mammals not listed by him, increases the total number to 106 from 120 hosts.

The genus itself may now be defined as *Taenioidea*:

Scolex with simple rostellum armed with a double row of very numerous and generally very small hammer-shaped hooks. Suckers armed or unarmed¹.

¹ Both Ransom (1909, p. 67) and Fuhrmann (1908, p. 42) omit to state in their diagnoses of the genus that the suckers are sometimes unarmed (e.g. *D. cesticillus* Mol.).

A single set of reproductive organs in each segment. Genital pores unilateral or alternating¹. Uterus persistent (*D. uterina* Fuhr.), or transient: in the latter case the eggs are either enclosed in egg-capsules or are scattered throughout the parenchyma (*D. rhynchota* Rans.). Eggs with thin transparent shells. Adults in mammals or birds. Larval stage a cysticeroid parasitic in molluscs or insects. Type species—*Davainea proglottina* (Davaine, 1860).

The species of the genus fall roughly into four classes, the division being based upon the armature of the suckers, and the position of the genital pores.

- Aa.* Genital pores unilateral, suckers armed.
- Ab.* Genital pores unilateral, suckers unarmed.
- Ba.* Genital pores alternating, suckers armed.
- Bb.* Genital pores alternating, suckers unarmed.

This division, while useful for dealing with a large number of species and for identification, cannot be regarded as wholly natural. The armature of the suckers is very transient and many of the species at present said to possess unarmed suckers may, when their life-histories are fully known, be found to lose their acetabular hooks at an early stage. Neither is the position of the genital pores a character altogether suitable for the characterisation of subgenera. *D. tetragona* (Mol.) possesses unilateral genital pores and *D. echinobothrida* (Még.) alternating pores in the adult form and unilateral in the young. The two species are closely related and no classification can be regarded as satisfactory which separates them. A character upon which a classification may perhaps be finally based is the behaviour of the uterus and the origin of the egg-capsules. A large number of species have egg-capsules each containing only one egg (e.g. *D. cesticillus* Mol., *D. corvina* Fuhr., *D. microcotyle* Skrj.), others possess from two (*D. celebensis* Jan., *D. friedbergi* Lins.) to 14 or 15 eggs per capsule (*D. oligorchidna* Fuhr., *D. salmoni* Stiles): in *D. insignis* Steudener (1877, p. 302)—“Der Uterus dehnt sich dann mehr und mehr aus und bildet schliesslich einen die ganze Mittelschichte des Gliedes einnehmenden Sack, in welchen die Eier durch eine körnige Substanz zu Conglomeraten verbunden sind,” and in *D. uniuterina* (Fuhrmann, 1909, p. 114)—“Der Uterus scheint sich nicht (oder sehr spät) in einzelne Parenchymkapseln aufzulösen, sondern als einheitlicher, stark gelappter Uterus bestehen zu bleiben,” while in *D. rhynchota* Ransom (1909, p. 14), distinctly states that the eggs are scattered singly throughout the parenchyma and are not enclosed in capsules. Beddard (1914, pp. 875 and 885) again considers the egg-capsules as formed by a number of parauterine organs. Unfortunately our knowledge of the behaviour of the uterus in this genus is not sufficiently extensive to allow of the utilisation of this character. Its structure and behaviour in many of the species are entirely unknown and in others but insufficiently described. All our knowledge of *D. difformis* (Rud.) is contained in a statement by Fuhrmann (1908, p. 45), “Rudolphi nannte diese Art

¹ Ransom (*loc. cit*) states the genital pores *occasionally* alternate.

T. difformis Rud. 1819; sie ist, wie er selbst sagt, identisch mit *Taenia brevicollis* Frölich. In der Rudolphischen Sammlung finden sich in Glas No. 1906 die Typen dieses Cestoden, deren Untersuchung gezeigt, dass es ein Vertreter des Genus *Davainea* ist. Ich behalte den Namen, zu welchem die Typen existieren, bei." *D. globocephala* Fuhr., *D. circumcincta* (Krabbe), *D. longicollis* (Mol.) are equally unknown, while of *D. australis* (Krabbe) only the armature of the head has been described. Under these circumstances any classification based upon characters other than those usually given (armature of scolex, position of genital pore) must of necessity exclude large numbers of species. I have therefore sub-divided the genus into the four divisions given above as the best available means of separating the greatest number of species into distinct classes with a view to facilitating identification and systematic manipulation. The classification of birds follows that of Sharp in the British Museum Handbook of Birds. The references given are other than are to be found in Fuhrmann's *Die Cestoden der Vögel*.

LIST OF SPECIES OF DAVAINEA AND THEIR HOSTS.

Mammalia.

PRIMATES.

Homo sapiens L.

D. asiatica (von Linstow, 1901); 1901 *a*, 982; 1901 *b*, 283.

D. formosana Akashi¹, 1917.

*D. madagascariensis*² (Davaine, 1870); Blanchard, 1891 *a*, 438; 1891 *b*, 613; 1897, 34; 1899 *a*, 200 and 215. Chevreau, 1891, 523. Davaine, 1877, liii and 922. Daniels, 1895; 1896, 1455. Grenet and Davaine, 1869, 233; 1870, 134. Holzberg, 1898, 174. Leuckart, 1891, 68. v. Linstow, 1901 *a*, 984. Parona, 1898, 106. Stevens, 1916, 329. Stiles, 1896 *b*, 55.

Galeopithecus volans.

D. lateralis Bourquin, 1906, 222.

RODENTIA.

Arvicanthys abyssinicus.

D. polycalceola v. Janicki, 1902. Parona, 1909, 2.

Arvicanthys pumilis Sparrm. var. *bechanae*.

D. trapezoides v. Janicki, 1904. Hungerbühler, 1910, 516.

Geomys breviceps Baird.

D. sp. Douthitt, 1915.

RODENTIA—continued.

Lepus arizonae.

D. retractilis Stiles, 1895. Stiles, 1895; 1896, 195. Blanchard, 1899 *a*, 207 and 215. Parona, 1898, 106.

Lepus melanotis.

D. salmoni Stiles, 1895. Stiles, 1895; Stiles, 1896, 198. Blanchard, 1899, 207 and 214.

Lepus sylvaticus.

D. salmoni Stiles, 1895. Stiles, 1896, 198. Blanchard, 1899 *a*, 207 and 214. Parona, 1898, 106.

Mus flavidus.

D. (?) gracilis v. Janicki, 1904, 779; 1906, 570.

Mus meyeri.

D. celebensis v. Janicki, 1902, 275.

Mus muschenbrocki.

D. polycalceola v. Janicki, 1902, 258; 1906, 543. Parona, 1909, 2.

Mus rajah.

D. blanchardi (Parona, 1898). Blanchard, 1899 *a*, 210 and 216. Parona, 1898, 103.

¹ If the rostellar hooks be thorn-shaped, this is more probably a *Dipylidium*.

² Synonym: *Taenia demarariensis* Daniels, 1895.

RODENTIA—continued.

Mus siporanus.*D. blanchardi* (Parona, 1898). Blanchard, 1899 *a*, 210 & 216. Parona, 1898, 103.*Mus variegatus*.*D. trapezoides* v. Janicki, 1904, 779; 1906, 568. Hungerbühler, 1910, 516.

INSECTIVORA.

Erinaceus albiventris.*D. voluta*¹ (v. Linstow, 1904). v. Linstow, 1904 *b*, 381. v. Janicki, 1906, 543.

INSECTIVORA—continued.

Erinaceus sp.*D. parva*² v. Janicki, 1904, 774; 1906, 540.

EDENTATA.

Manis pentadactyla.*D. contorta* Zschokke, 1895, 634. Blanchard, 1899 *a*, 215. Parona, 1898, 106. Stiles, 1896, 195.

Aves.

ACCIPITRIFORMES.

Buteo desertum (Daud.).*D. sphaeroides* Clerc, 1902.*Buteo menetriesi* Bogd.*D. sphaeroides* Clerc, 1902.*Entolmaëtus fasciatus* (Vieill.).*D. hertwigi* Mola, 1907.

ANSERIFORMES.

Anas bochas domesticus.*D. anatina*³ Fuhrmann, 1909. Lühe, 1910, 52. Skrjabin, 1914 *a*, 68. Stiles, 1896 *b*, 53.? *D. crassula* (Rudolphi, 1919), Clerc non Fuhrmann. Stiles, 1896 *b*, 53.*D. microcotyle* Skrjabin, 1914 *a*, 69.*Anas* sp.*D. cyrtus* Skrjabin, 1914 *a*, 67.

APTERYGIFORMES.

Crypturus noctivagus (Neu.).*D. crypturi* Fuhrmann, 1909.*Crypturus* sp.*D. capillaris* Fuhrmann, 1909.*Nothura media* (Spix.).*D. elongata* Fuhrmann, 1909.*Rhynchotus rufescens* (Temm.).*D. elongata* Fuhrmann, 1909.*D. oligocantha* Fuhrmann, 1909.

APTERYGIFORMES—continued.

Tinamus sp.*D. elongata* Fuhrmann, 1909.*D. oligocantha* Fuhrmann, 1909.

ARDEIFORMES.

Garzetta garzetta (L.).*D. circumcincta* (Krabbe, 1869). Blanchard, 1899 *a*, 215.

CASUARIIFORMES.

Casuarius uniappendiculatus Blyth.*D. sp. nov.* Vevers, 1920, 408.*D. sp. nov.* Vevers, 1920, 408.*Dromaeus novae-hollandiae* Lath.*D. australis* (Krabbe, 1869). Blanchard, 1899 *a*, 215. Johnston, 1912, 106. Stiles, 1896 *b*, 54.

CHARADRIIFORMES.

Himantopus leucocephalus Gould.*D. himantopodis* Johnston, 1911. Johnston, 1911, 75; 1912, 107.*Totanus fuscus* (L.).*D. minuta* Cohn, 1901. Lühe, 1910, 52.

COCCYGES.

Chrysococcys klassi (Steph.).*D. sp.*⁴ Parona, 1909.¹ Considered by v. Janicki (1906, p. 543) as possibly identical with his own species *D. parva*.² This species, together with the closely allied *D. voluta* (v. Linstow), is placed by Janicki (1906, p. 543) in the genus *Davainea*, but the absence of hooks on the scolex, the passage of the genital canals dorsal to the nerve and excretory vessels, the position of the testes lateral and posterior to the female organs, and the enclosure of the eggs single in egg-capsules seem rather to refer it to the genus *Oochoristica*.³ Synonyms: *D. crassula* (Rud.) Wolffhügel, 1899.⁴ This species is considered by Parona (1909) as possibly identical with *D. difformis* Rud. or *D. mutabilis* Rüther.

COCYGES—continued.

- Corythaeola cristata* (Vieill.).
D. calcarea Fuhrmann, 1909.
D. undulata Fuhrmann, 1909.
Cuculus canorus L.
*D. difformis*¹ (Rudolphi, 1819).
Gallerix porphyreolophus (Vig.).
D. leptosoma (Diesing, 1850).
Schizorhis concolor (Smith).
D. sp. inq. Vevers, 1920, 408.
Turacus buffoni (Vieill.).
D. macrocirrosa Fuhrmann, 1909. Skrjabin, 1914 a, 70.

COLUMBRIFORMES.

- Caloenas nicobarica* (L.).
D. paucitesticulata Fuhrmann, 1909. Vevers, 1920, 408.
Columba livia Bonn.
*D. crassula*² (Rudolphi, 1819). Fuhrmann non Clerc. Blanchard, 1899 a, 215. Fuhrmann, 1909, 104. Krabbe, 1869, 345; 1879, 15. ? Stossich, 1895, 43.
*D. crassula*² (Rudolphi, 1819). Clerc non Fuhrmann. Blanchard, 1891 a, 436. Clerc, 1906, 725. Krabbe, 1882, 363; 1879, 15. Railliet, 1893, 306. Stiles, 1896 b, 53.
Columba palumbus L.
D. columbae Fuhrmann, 1909.
Columba sp.
D. cryptacantha Fuhrmann, 1909.
D. spiralis Baczynska, 1914, 198.
Globicera oceanica (Less.).
D. insignis (Steudener, 1877).
Goura albertisi Salvad.
D. goura Fuhrmann, 1909.

¹ Synonym: *Taenia brevicollis* Frolich.

² Under the name *D. crassula* (Rud., 1819) two different forms have been described. The description of the one form was by Fuhrmann (1909, p. 104) from the study of Rudolphi's type species and therefore must be regarded as defining the species. The other form described under the name *D. crassula* (Rud.) by Stiles (1896 b, p. 53), Railliet (1893, p. 306) and Clerc (1906, p. 725), and under that of *D. columbae* (Zed.) by Blanchard (1891 a, p. 436) differs from the type species in having unilateral genital pores, armed suckers, and rostellar hooks of half the size of those seen by Fuhrmann. Two species seem thus to have been confused together under the one name. Clerc's form further differs from those seen by Stiles and Railliet in having 400 rostellar hooks instead of 70. Fuhrmann (1908, pp. 74 and 44) considers *Alyselminthus columbae* Zeder (1800) and *Halysis columbae* Zeder (1803) as possible synonyms of *D. crassula* Rud. This Cestode was first described by Rudolphi (1810, p. 94) under the name *Taenia sphencephala*, but as this, according to Fuhrmann's (1906, p. 449) study of the type species, is a *Hymenolepis*, *Alyselminthus columbae* and *Halysis columbae* cannot possibly be synonyms of *D. crassula*.

³ Synonyms: *Taenia calva* Baird, 1853; *T. microps* Diesing, 1857; *T. tumens* Mehlis.

⁴ Synonym: *Taenia lineata* Rudolphi ex parte.

COLUMBRIFORMES—continued.

- Goura coronata* (L.).
D. goura Fuhrmann, 1909. Vevers, 1920, 408.
Leucosarcia picata (Lath.).
D. sp. Johnston, 1912, 106.
Turtur decipiens (Fin. and Hartl.).
D. cryptacantha Fuhrmann, 1908.
Turtur turtur (L.).
*D. crassula*² (Rudolphi, 1819). Clerc non Fuhrmann. Blanchard, 1891 a, 436. Stiles, 1896 b, 53.
D. micracantha Fuhrmann, 1909.

CORACIIFORMES.

- Buceros seratogynia*.
D. emperus Skrjabin, 1914 a, 69.
Colius leucotis affinis Shell.
D. wernerii Klaptoch, 1908, 281. Fuhrmann, 1912, 192.
Podager nacunda (Vieill.).
D. magnicoronata Fuhrmann, 1909. Skrjabin, 1914 a, 70.

GALLIFORMES.

- Caccabis chukar* Gray.
D. nov. sp. Vevers, 1920, 408.
Caccabis petrosa (Gm.).
D. circumvallata (Krabbe, 1869). Mola, 1907 b, 126; ? 1912, 440.
Caccabis saxatilis (Wolf and Meyer).
D. crassula (Rudolphi, 1819). Clerc non Fuhrmann. Stiles, 1896 b, 53.
*D. urogalli*³ (Modeer, 1790). Morell, 1895, 16.
Coturnix coturnix (L.).
*D. circumvallata*⁴ (Krabbe, 1869). ? Mola, 1912, 440. Stiles, 1896 b, 47. Stossich, 1895, 42.

GALLIFORMES—continued.

D. polyuterina Fuhrmann, 1909.

Coturnix communis.

D. circumvallata (Krabbe, 1869). Blanchard, 1891 *a*, 434.

*D. pluriuncinata*¹ (Crety, 1890). Blanchard, 1891 *a*, 434. Crety, 1890, 10. Fuhrmann, 1908, 43. Mola, 1912, 432. Stiles, 1896 *a*, 47.

Crax alector (L.).

D. leptacantha Fuhrmann, 1909.

Crax fasciolata Spix.

D. leptacantha Fuhrmann, 1909.

Crax sp.

D. leptacantha Fuhrmann, 1909.

Francolinus adpersus Waterh.

D. provincialis v. Linstow, 1909, 75.

Francolinus clappertoni Childr.

D. clavicirrosa Fuhrmann, 1909 *b*, 13.

Gallus gallus domesticus.

*D. cantaniana*² (Polonio, 1860). Blanchard, 1899 *a*, 215; 1899, 480.

*D. cesticillus*³ (Molin, 1858). Aekert, 1918, 41. ? Dujardin, 1845, 586. Guberlet, 1916 *b*, 37; 1916 *a*, 232. ? Holzberg, 1898, 182. Krabbe, 1879, 13. Meggitt, 1916, 399. Railliet, 1893, 305. Setti, 1893, 20. Sweet, 1910, 242.

D. cohni Baczynska, 1914, 196.

D. dubius Meggitt, 1916, 391.

*D. echinobothrida*⁴ (Méglin, 1880). Guberlet, 1916 *b*, 36. Ransom, 1904, 55; 1905, 280.

*D. longicollis*⁵ (Molin, 1858). Ariola, 1900, 432. Blanchard, 1894, 701. Fuhrmann, 1908, 43. v. Linstow, 1878, 123. Railliet, 1893, 329.

D. mutabilis Rüther, 1901.

D. penetrans Baczynska, 1914, 192.

D. proglottina (Davaine, 1860). Blanchard, 1899 *a*, 214. Dujardin, 1843, 342; 1845, 630. Hungerbühler, 1910, 519. Magalhães, 1898, 443. Railliet, 1893, 305. Railliet et Lueet, 1892, 105.

¹ Synonym: *D. circumvallata* (Krabbe) of Morell, 1895.

² Synonym: *D. oligophora* Magalhães, 1898.

³ Synonym: *Taenia infundibuliformis* Goeze of Dujardin, 1845.

⁴ Synonyms: *Taenia botrioplitis* ex parte, *Davainea parachinobothrida* Mag., 1898. For full synonymy and literature see Ransom (1904, p. 64) or (1905, p. 278).

⁵ Synonyms: *Dibothrium longicollis* (Molin), *Bothriocephalus longicollis* (Molin), *Bothriotaenia longicollis* (Molin).

⁶ See footnote 3, p. 12.

GALLIFORMES—continued.

D. proglottina var. *dublanensis* Kowalewski, 1905.

*D. tetragona*⁴ (Molin, 1858). Aekert, 1919, 28. Guberlet, 1916 *a*, 233; 1916 *b*, 34. Setti, 1893, 20. Sweet, 1910, 243. Rätz, 1900, 230.

D. varians Sweet, 1910, 243.

D. volzi Fuhrmann, 1905.

Gallus sonnerati Temm.

*D. cesticillus*³ (Molin, 1858).

Lagopus scoticus (Lath.).

*D. cesticillus*³ (Molin, 1858). Shipley, 1908, 263; 1909 *a*, 357.

*D. urogalli*⁶ (Modeer, 1790). Shipley, 1908, 264; 1909 *a*, 351; 1909 *b*, 368.

Lyrurus tetrix (L.).

D. retusa Clere, 1903. Shipley, 1909 *b*, 366.

*D. urogalli*⁶ (Modeer, 1790). Morell, 1895, 16. Shipley, 1909 *b*, 366.

Meleagris gallopavo L.

*D. cantaniana*² (Polonio, 1860). Blanchard, 1899 *a*, 215. Fuhrmann, 1908, 104.

D. cesticillus (Molin, 1858).

Numida meleagris L.

? *D. cantaniana*² (Polonio, 1860).

Numida ptilorhyncha Licht.

D. nana Fuhrmann, 1912, 185.

D. numida Fuhrmann, 1912, 182.

D. paucisegmentata Fuhrmann, 1909 *b*, 15.

D. pintneri Klaptocz, 1906. Fuhrmann, 1909 *b*, 12; 1912, 191.

Pavo cristatus L.

D. ceylonica Baczynska, 1914, 190.

Penelope obscura Illig.

D. penelopina Fuhrmann, 1909.

Perdix perdix (L.).

D. globirostris Fuhrmann, 1909.

D. polyuterina Fuhrmann, 1909.

Perdix sp.

D. campanulata Fuhrmann, 1909.

D. circumvallata (Krabbe, 1869).

GALLIFORMES—continued.

- D. laticanalis* Skrjabin, 1914 a, 66.
Phasianus colchicus L.
*D. cantaniana*¹ (Polonio, 1860). Fuhrmann, 1908, 104.
*D. friedbergi*² (v. Linstow, 1878). Baczyńska, 1914, 200.
Phasianus sp.
D. multicapsulata Baczyńska, 1914.
Tetrao urogallus L.
*D. globocaudata*³ Cohn, 1900. Cohn, 1900, 95. Neslobinsky, 1911, 436. Shipley, 1909 b, 368.
D. urogalli (Modeer, 1790). Morell, 1895, 16. Shipley, 1909 b, 368. Cohn, 1900, 95.
Tetraogallus himalayensis Gray.
*D. urogalli*⁴ (Modeer, 1790). Morell, 1895, 16.

GRUIFORMES.

- Cariama cristata* L.
D. brachyrhyncha (Creplin, 1853).

PASSERIFORMES.

- Cacicus affinis* Swains.
D. globocephala Fuhrmann, 1909.
Conopophila albigularis (Gould).
D. conopophilae Johnston, 1912, 110.
Corvus coronoides Vig. and Horsf.
D. sp. Johnston, 1912, 112.
Corvus culminatus Sykes.
*D. corvina*⁵ Fuhrmann, 1905. Fuhrmann, 1908, 46; 1911, 251.
Corvus macrorhynchus Wagl.
D. corvina Fuhrmann, 1905
Entomyza cyanotis (Lath.).
D. conopophilae Johnston, 1912, 111.
Fringilla coelebs L.
D. sp. inq. Ververs, 1920, 408.
Galerita macrorhyncha Tristr.
D. galeritae Skrjabin, 1914 a, 70.
Macrocorax fuscicapillus (Gray).
*D. corvina*⁵ Fuhrmann, 1905. ? Fuhrmann, 1908, 46; 1911, 251.

PASSERIFORMES—continued.

- Manucodia chalybeata* Penn.
D. paradisea Fuhrmann, 1909.
Merula merula (L.).
D. spinosissima (v. Linstow, 1894, 336). Blanchard, 1899 a, 206 and 214.
Mino dumonti Less.
*D. corvina*⁵ Fuhrmann, 1905. ? Fuhrmann, 1908, 46; 1911, 251.
Oriolus galbula (L.).
D. compacta Clerc, 1906. Neslobinsky, 1911, 436.
D. frontina (Dujardin, 1845). Stiles, 1896 b, 54.
Philemon citreigularis (Gould).
D. conopophilae Johnston, 1912, 111.
Rupicola rupicola (L.).
D. uniuterina Fuhrmann, 1909.
Sphecotheres maxillaris (Lath.).
D. sphecotheridis Johnston, 1914, 106.

PICIFORMES.

- Celeus elegans* (P. L. S. Müll.).
D. longispina Fuhrmann, 1909.
D. lutzi Parona, 1901. Fuhrmann, 1909, 112.
Ceophloeus lineatus (L.).
D. longispina Fuhrmann, 1909.
D. cruciata (Rudolphi, 1819).
Colaptes auratus.
D. rhynchota Ransom, 1909, 10.
D. comitata Ransom, 1909, 15.
Colaptes campestris (Vieill).
D. frontina (Dujardin, 1845).
Drendrocoptes medius (L.).
D. frontina (Dujardin, 1845). Neslobinsky, 1911, 436.
Drendrocopus major (L.).
D. frontina (Dujardin, 1845). Stiles, 1896 b, 54.
Gecinus canus (Gm.).
D. cruciata (Rudolphi, 1819).
Gecinus viridis (L.).
D. frontina (Dujardin, 1845).

¹ Synonym: *D. oligophora* Magalhães 1898.

² Synonyms: ? *Taenia agama* Mégnin; *T. cesticillus* var. *phasianorum* Neumann; ? *T. infundibuliformis* var. *phasianorum* Mégnin; *Davainea guevillensis* Mégnin.

³ Not *D. globocaudata* Cohn 1901 as given by Fuhrmann, 1908.

⁴ Synonyms: *Taenia calva* Baird, 1853; *T. microps* Diesing 1857; *T. tumens* Mehlis.

⁵ Synonym: *D. polycalcarea* v. Linstow, 1906.

PICIFORMES—*continued*.

- Melanerpes erythrocephalus* (L.).
D. comitata Ransom, 1909, 15.
D. rhynchota Ransom, 1909, 10.
Picus martius L.
D. frontina (Dujardin, 1845). Neslobinsky, 1911, 436.
Picus sp.
D. cruciata (Rudolphi, 1819). Fuhrmann, 1908, 46; 1909, 111.
D. longispina Fuhrmann, 1909.
D. frontina (Dujardin, 1845).
D. lutzi Parona, 1901

PSITTACIFORMES.

- Ara aureicollis* Cass.
D. leptosoma (Diesing, 1850).
Ara macao (L.).
D. leptosoma (Diesing, 1850).
Ara maracana (Vieill.).
D. leptosoma (Diesing, 1850).
Ara macavuanna (Gm.).
D. leptosoma (Diesing, 1850).
Ara nobilis (L.).
D. leptosoma (Diesing, 1850).
Ara severa (L.).
D. leptosoma (Diesing, 1850).
Cacatua galerita (Lath.).
D. cacatuina Johnston, 1912, 109.
Cacatua roseicapilla Vieill.
D. leptosoma (Diesing).
Cacatua triton macrolopha (Rosenb.).
D. psittacea Fuhrmann, 1911, 255.
Chrysotis purpurca.
D. leptosoma (Diesing, 1850).
Conurus guaronba (Gm.).
D. leptosoma (Diesing, 1850).
Electus pectoralis aruensis (Gray).
D. microscolecina Fuhrmann, 1909. Fuhrmann, 1911, 254.
D. oligorchidna Fuhrmann, 1911, 256.

PSITTACIFORMES—*continued*.

- Electus rosatus* (Müll.).
D. microscolecina Fuhrmann, 1909. Fuhrmann, 1911, 254.
Lorius garrulus (L.).
D. macroscolecina Fuhrmann, 1909.
Pionopsittacus pileatus (Scop.).
D. macroscolecina Fuhrmann, 1909.
Pionus fuscus (Müll.).
D. leptosoma (Diesing, 1850).
Psittacus erythacus L.
D. crassula (Rudolphi, 1819). Clerc non Fuhrmann. Stiles, 1896 b, 53.
*D. leptosoma*¹ (Diesing, 1850.) Stiles, 1896 b, 54.
Psittacus sp.
D. macroscolecina Fuhrmann, 1909.
Trichoglossus cyanogrammus nigrogularis Gray.
D. aruensis Fuhrmann, 1911, 255.

PTEROCLIDIFORMES.

- Pteroclidurus namaquus* (Gm.).
D. leptotrachela Hungerbühler, 1910, 512.

RHEIFORMES.

- Rhea americana* L.
 ? *D. struthionis* (Parona, 1885).

STRUTHIONIFORMES.

- Struthio australis* Gurn.
D. linstowi Meggitt (present paper). Hungerbühler, 1910, 511.
Struthio camelus L.
D. struthionis (Parona, 1885).
Struthio masaicus Neu.
D. beddardi Meggitt (present paper).
D. struthionis (Parona, 1885). Beddard, 1915, 589.
Struthio molybdophanos Reichen.
D. linstowi Meggitt (present paper). Beddard, 1915, 589. Blanchard, 1899 a, 206. v. Linstow, 1893, 447.

Reptilia.

- Lacerta muralis*.
D. hertwigi Mola, 1907 a, 575, cysticeroid.

¹ Synonyms: *Taenia longissima* Goeze, *T. filiformis* Rudolphi.

Insecta.

- Musca domestica* L. *D. cesticillus* Ackert, 1918, 41. Guberlet,
D. tetragona (Molin, 1858), cysticeroid. 1916 a, 233.
 Ackert, 1919, 28. Guberlet, 1916 a, 233.

Mollusca.

- Limax agrestis* L. *Limax variegatus* Drap.
D. proglottina (Davaine, 1870), cysticeroid. *D. proglottina* (Davaine, 1870), cysticeroid.
Limax cinereus Lister.
D. proglottina (Davaine, 1870), cysticeroid.

Hosts unknown.

- D. appendiculata* Fuhrmann, 1909. *D. echinata* Fuhrmann, 1909.

Key to Species of Davainea.

Genital pores unilateral, suckers armed	<i>Aa</i>
Genital pores unilateral, suckers unarmed	<i>Ab</i>
Genital pores alternate, suckers armed	<i>Ba</i>
Genital pores alternate, suckers unarmed	<i>Bb</i>

Aa.

1. Eggs singly in the parenchyma (2)
 Eggs in groups in capsules (13)
2. Proglottides with lateral appendages *D. appendiculata* Fuhr.
 Proglottides without lateral appendages. (3)
3. Rostellum with accessory spines (4)
 Rostellum without accessory spines. (5)
4. Rostellar hooks in two rows *D. sphecotheridis* John.
 Rostellar hooks in form of a rosette with eight limbs *D. rhynchota* Rans.
5. Testes 2 *D. contorta* Zsch.
 Testes more than two. (6)
- +
 6. Cirrus-sac nearly reaching aporal margin (7)
 Cirrus-sac not passing centre of proglottis (8)
7. Rostellar hooks 400, 15 μ long *D. compacta* Clerc.
 Rostellar hooks 300, 6–7 μ long *D. sphaeroïdes* Clerc.
 Rostellar hooks 200, 14–16 μ long *D. cruciata* Rud.
 Rostellar hooks 160–180, 9–10 μ long *D. numida* Fuhr.
8. Rostellar hooks 400 *D. compacta* Clerc.
 Rostellar hooks 200 (9)
 Rostellar hooks 120 or less (10)
9. Rostellar hooks 14–16 μ long *D. cruciata* Rud.
 Rostellar hooks 22–23 μ long *D. paradisea* Fuhr.
10. Testes 100 (11)
 Testes 50 or less (12)
11. Rostellar hooks 12 μ long *D. retractilis* Stiles
 Rostellar hooks 22–23 μ long *D. paradisea* Fuhr.

KEY TO SPECIES OF DAVAINEA—continued.

12. Rostellar hooks 90 *D. madagascariensis* Dav.
 Cirrus-sac not passing nerve-cord; rostellar hooks
 80 *D. corvina* Fuhr.
 Cirrus-sac reaching lateral excretory vessel; rostellar hooks 120 *D. gracilis* Jan.
13. Accessory hooks posterior to rostellum *D. frontina* Duj.
 Accessory hooks absent (14)
14. Testes extending laterally beyond nerve. *D. penelopina* Fuhr.
 Testes not extending laterally beyond nerve (15)
- ++
 15. Cirrus-sac not reaching excretory vessels (16)
 Cirrus-sac reaching or passing excretory vessels (24)
 Cirrus-sac nearly reaching to aporal excretory vessels *D. sphaeroides* Clerc.
- ++++
 16. Egg-capsules extending laterally beyond excretory vessels. (17)
 Egg-capsules not extending laterally beyond excretory vessels (20)
- ++++
 17. Egg-capsules containing 2–3 eggs (18)
 Egg-capsules containing more than 5 eggs (19)
18. Receptaculum seminis present *D. friedbergi* v. Lins.
 Receptaculum seminis absent *D. multicapsulata* Fuhr.
19. Rostellar hooks 140, 5 μ long *D. cyrtus* Skr.
 Rostellar hooks 120, 10 μ long *D. ceylonica* Bacz.
 Rostellar hooks 100, 6–9 μ long *D. tetragona* (Molin)
- +++
 20. Three layers of longitudinal muscles *D. spiralis* Bacz.
 Two layers of longitudinal muscles (21)
21. Testes 5–6 *D. oligorchidna* Fuhr.
 Testes more than 12 (22)
22. Rostellar hooks 80 *D. comitata* Rans.
 Rostellar hooks more than 150. (23)
23. Egg-capsules contain 3–6 eggs *D. galeritae* Skrj.
 Egg-capsules contain 6–12 eggs *D. microcolecina* Fuhr.
 Egg-capsules contain 10–14 eggs *D. psittacea* Fuhr.
 ? *D. wernerii* Klap.
24. 20 eggs per egg-capsule (25)
 12 eggs or less per egg-capsule (26)
25. Rostellar hooks 200, testes 20–30 *D. pintneri* Klap.
 Rostellar hooks 120, testes 35–40 *D. gracilis* Jan.
 Rostellar hooks 70, testes 60 *D. leptosoma* (Dies.).
26. Testes 15 or less. *D. trapezoides* Jan.
 Testes 20 or more (27)
27. Rostellar hooks 8 μ long *D. crassula* (Rud.) Clerc.
 Rostellar hooks 10 μ long, egg-capsules extending laterally to excretory vessels *D. volzi* Fuhr.
 Rostellar hooks 10–13 μ long, egg-capsules not extending laterally to excretory vessels *D. microcolecina* Fuhr.

KEY TO SPECIES OF DAVAINEA—continued.

- Rostellar hooks 14μ long, egg-capsules extending
laterally to excretory vessels *D. clavicirrosa* Fuhr.
? *D. gracilis* Jan.
- + *D. cantaniana* Polonio and *D. blanchardi* Parona here.
- ++ *D. capillaris* Fuhr., *D. penetrans* Bacz. here.
- +++ *D. circumvallata* (Krabbe)—when young—and *D. echinobothrida* (Még.)—
when with unilateral genital pores—here.
- ++++ *D. cohnii* Bacz.
- +++++ *D. insignis* Steud., *D. urogalli* (Modeer).

Ba.

1. Proglottides less than 10 (2)
Proglottides numerous (5)
2. Testes 4 *D. himantopodis* John.
Testes 10 or more (3)
3. Testes lateral or anterior *D. varians* Sweet
Testes in a double row along posterior margin of proglottis (4)
4. Rostellar hooks $60, 7-8\mu$ long *D. dubius* Meggitt
Rostellar hooks $80-95, 5-7.5\mu$ long *D. proglottina* (Dav.)
5. Cirrus-sac not reaching excretory vessel (6)
Cirrus-sac reaching or passing excretory vessel (8)
6. Egg-capsules contain one egg *D. polycalceola* Jan.
Egg-capsules contain several eggs (7)
7. Female organs in poral half of proglottis *D. leptotrachela* Hung.
Female organs in centre of proglottis (7a)
- 7a. Testes 20-30 *D. echinobothrida* (Még.).
Testes 10-15 *D. cohnii* Bacz.
8. Rostellar hooks 8μ or less (9)
Rostellar hooks 11μ or more (10)
9. Suckers armed with 3 rows of hooks *D. hertwigi* Mola.
Suckers armed with 6-8 rows of hooks *D. pluriuncinata* Crety
10. Genital pore anterior, in birds *D. retusa* Clerc.
Genital pore anterior, in birds *D. hertwigi* Mola.
Genital pore posterior, in rodents *D. salmoni* Stiles

Ab.

1. Proglottides 5 *D. paucisegmentata* Fuhr.
Proglottides numerous (2)
2. A wavy line of accessory hooks present *D. echinata* Fuhr.
Accessory hooks absent (2a)
- 2a. Rostellar hooks thorn-shaped *D. formosana* Akashi.
Rostellar hooks T-shaped (3)
- + 3. Eggs singly in parenchyma (4)
Egg-capsules contain several eggs (5)
4. Rostellar hooks $14-16\mu$ long *D. longispina* Fuhr.
Rostellar hooks $20-30\mu$ long *D. struthionis* (Par.)
Rostellar hooks $25-28\mu$ long *D. undulata* Fuhr.

KEY TO SPECIES OF DAVAINEA—continued.

5. Rostellar hooks 75–88 μ long (6)
 Rostellar hooks 25–28 μ long *D. undulata* Fuhr.
 Rostellar hooks less than 20 μ long (7)
- ++++
6. Cirrus-sac reaching excretory vessels *D. linstowi* Meggitt
 Cirrus-sac not reaching excretory vessels *D. beddardi* Meggitt
7. Testes forming a ring round the female organs *D. cryptacantha* Fuhr.
 Testes lateral and posterior to female organs. (8)
8. Genital cloaca with powerful sphincter muscle *D. macrocirrosa* Fuhr.
 Sphincter muscle of genital cloaca weak or absent (9)
- ++
9. Testes less than 15 (10)
 Testes more than 20 (11)
10. Rostellar hooks 120, 9–10 μ long *D. paucitesticulata* Fuhr.
 Rostellar hooks 300, 14–16 μ long *D. calcarea* Fuhr.
 Rostellar hooks ?, 6–7 μ long *D. crypturi* Fuhr.
11. Rostellar hooks 9 μ long or less (12)
 Rostellar hooks 12 μ or more (14)
12. Egg-capsules extending laterally beyond excretory vessel. *D. crypturi* Fuhr.
 Egg-capsules not extending laterally beyond excretory vessel (13)
13. Cirrus-sac reaching excretory vessel *D. goura* Fuhr.
 Cirrus-sac not reaching excretory vessel *D. lateralis* Bourq.
14. Rostellar hooks 500, 14 μ long *D. provincialis* v. Linstow
 Rostellar hooks 350 or less (15)
- +++
15. Egg-capsules contain 4–5 eggs *D. lateralis* Bour.
 Egg-capsules contain 8 eggs or more (16)
16. Testes 20 (17)
 Testes 50 or more (18)
17. Rostellar hooks 180–200 *D. aruensis* Fuhr.
 Rostellar hooks 350 *D. macroscolecina* Fuhr.
18. Genital pore anterior *D. globirostris* Fuhr.
 Genital pore half-way. *D. leptacantha* Fuhr.
 Genital pore posterior *D. uniuterina* Fuhr.
- +
- D. micracantha* Fuhr. here.
- ++
- D. lutzi* Parona here.
- +++
- D. lutzi* Parona here.
- ++++
- D. lateralis* Bourq., *D. macroscolecina* Fuhr. here.

Bb.

1. Rostellar hooks absent (2)
 Rostellar hooks present (3)
2. Length 17 mm., genital pore ventral to proglottis margin *D. voluta* (v. Linstow)
 Length 21 mm., genital pore marginal *D. parva* v. Jan.
3. Rostellum longer than broad, 40 hooks, 27 μ long *D. campanulata* Fuhr.
 Rostellum broader than long (4)

KEY TO SPECIES OF DAVAINEA—continued

- ++
 4. Eggs singly in parenchyma (5)
 Egg-capsules contain several eggs (12)
- +
 5. Rostellar hooks 34, 21–23 μ long *D. oligocantha* Fuhr.
 Rostellar hooks over 100, under 18 μ long (6)
 6. Testes less than 20 (7)
 Testes more than 20 (9)
 7. Rostellar hooks 14–16 μ long *D. anatina* Fuhr.
 Rostellar hooks 9 μ long (8)
 8. Length .85 mm. *D. minuta* Cohn
 Length 150–200 mm. *D. magnicoronata* Fuhr.
 9. Rostellar hooks 7–11 μ long, testes 20–30 (10)
 Rostellar hooks 16–18 μ long, testes 40–50 (11)
 10. Cirrus-sac .24 mm. long *D. columbae* Fuhr.
 Cirrus-sac .15 mm. long *D. cesticillus* (Molin)
 11. Egg-capsules extend laterally beyond excretory
 vessels *D. polyuterina* Fuhr.
 Egg-capsules not extending laterally beyond ex-
 cretory vessels *D. nana* Fuhr.
 12. Testes less than 20 (7)
 Testes more than 20 (13)
- ++
 13. Rostellar hooks 70 *D. crassula* (Rud.) Fuhr.
 Rostellar hooks over 150 (14)
 Rostellar hooks 800 *D. circumvallata* (Krabbe)
 14. Rostellar hooks 27 μ long *D. mutabilis* R  th.
 Rostellar hooks 16 μ long (15)
 15. 3–4 eggs per egg-capsule *D. laticanalisis* Skrj.
 8–12 eggs per egg-capsule *D. elongata* Fuhr.
- + *D. microcotyle* Skrj.
 ++ *D. spinosissima* v. Linstow.

(The literature of the following species not being accessible to me, they are not included in the above key: *D. proglottina* var. *dublanensis* Kowalewski, 1905; *D. cacatuina* Johnston, 1912; *D. conopophilae* Johnston, 1912; and *D. vagintivasus* Skrjabin, 1914.)

In addition, lack of adequate description prevents the following species from being included: *D. asiatica* von Linstow, 1901; *D. australis* (Krabbe, 1869); *D. brachyrhnycha* (Creplin, 1853); *D. celebensis* von Janicki, 1902; *D. circumcincta* (Krabbe, 1869); *D. difformis* (Rudolphi, 1819); *D. emperus* Skrjabin, 1914; *D. globocephala* Fuhrmann, 1909; *D. longicollis* (Molin, 1858); *D. macrocirrosa* Fuhrmann, 1909; *D. sp.* Parona, 1909; *D. sp.* Johnston, 1912; *D. sp.* Johnston, 1912; *D. sp.* Douthitt, 1916; *D. sp.* Vevers, 1920; *D. sp.* Vevers, 1920; *D. sp.* Meggitt (present paper).

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EXPLANATION OF PLATE I.

REFERENCE LETTERS.

c. Cirrus-sac. *e.* Egg. *l.m.* Inner longitudinal muscles. *l'.m'.* Outer longitudinal muscles. *ov.* Ovary. *r.* Rostellum. *r.r.* Rostellar ridge. *r.s.* Receptaculum seminis. *s.* Sucker. *t.* Testes. *t.m.* Transverse muscles. *u.* Uterus. *v.* Vagina. *v.exc.* Ventral excretory vessel. *w.* Wall of egg-capsule. *w'.* Inner wall of egg-capsule.

PLATE I.

Fig. 1. *Cotugnia brotogerys* Meggitt. Scolex.

Figs. 2-4. *Davainea beddardi* n. sp.

Fig. 2. Scolex.

Fig. 3. Musculature of proglottis from centre of strobilus.

Fig. 4. Musculature of proglottis from anterior portion of strobilus.

Figs. 5-7. *Davainea* sp.

Fig. 5. Musculature of proglottis.

Fig. 6. Transverse section of egg-sac containing eggs.

Fig. 7. Proglottis with genital organs.

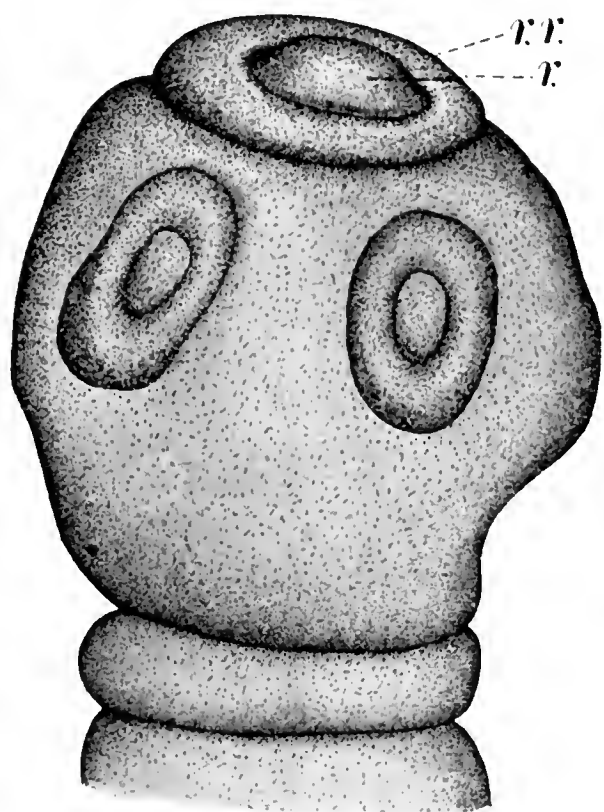


Fig. 1

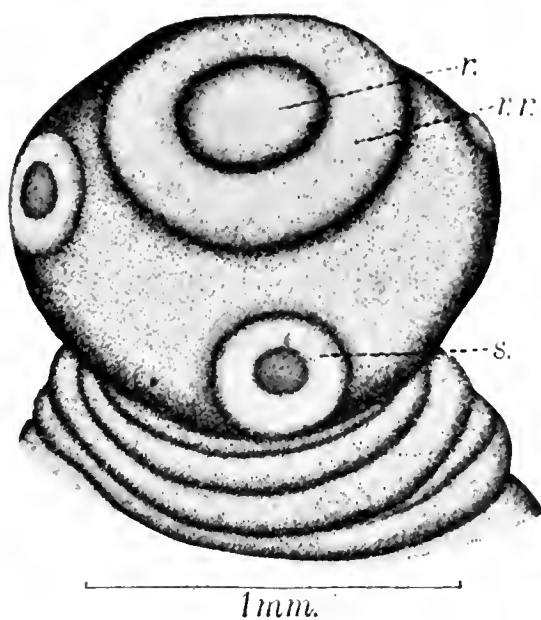


Fig. 2

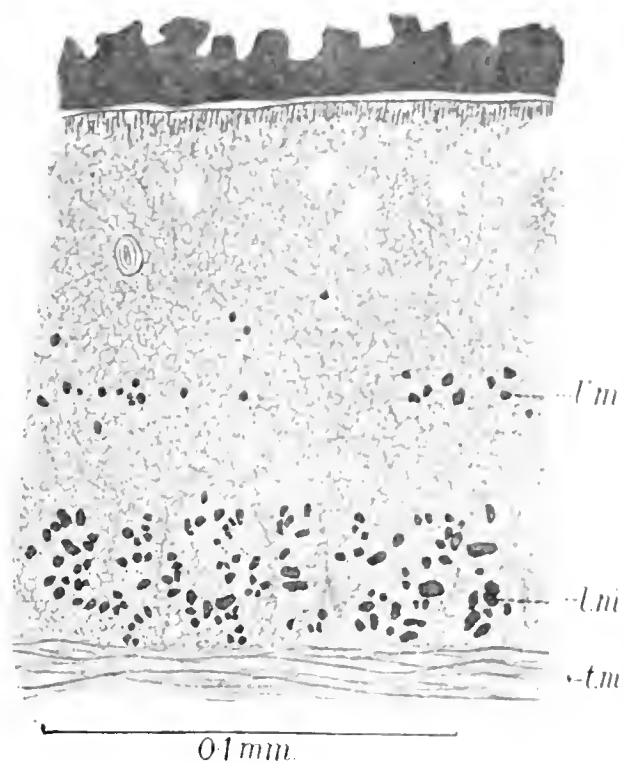


Fig. 3

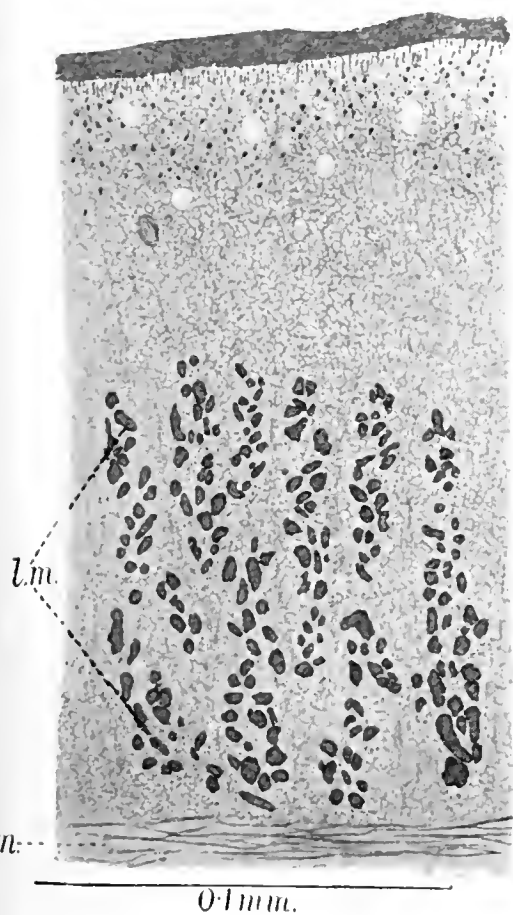


Fig. 4

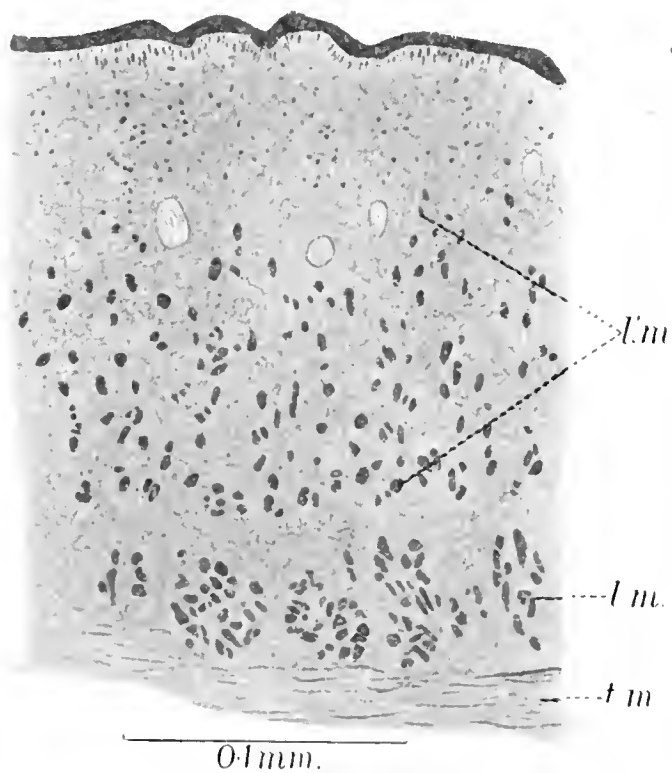


Fig. 5



Fig. 6

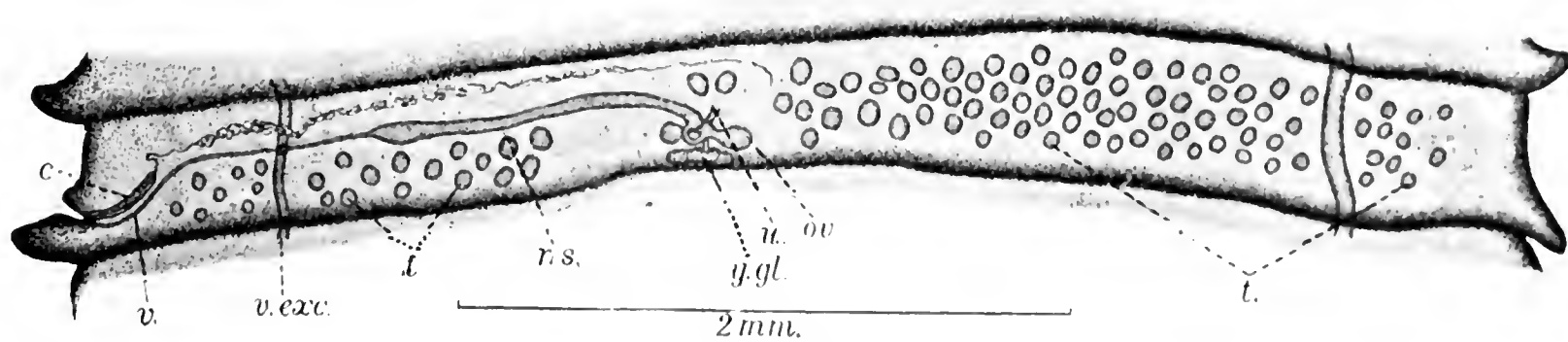


Fig. 7

A BACILLARY INFECTION OF THE COPULATORY APPARATUS OF *PEDICULUS HUMANUS*.

BY J. A. ARKWRIGHT AND A. BACOT.

(*From the Lister Institute of Preventive Medicine.*)

IN the paper on the association of *Rickettsia* with Trench Fever, by Arkwright, Bacot and Duncan (1919) mention is made of a bacillary infection of the excreta and guts of lice (*Pediculus humanus*). A Gram-negative cocco-bacillus was isolated in pure culture. It was non-motile, fermented glucose, mannite and lactose very slowly, and formed acid and clot in milk in about 14 days.

It was not pathogenic for guinea-pigs. When plated from pure culture, rough and smooth colonies were formed similar to those which are known to occur in bacilli of the *B. coli* group.

Continuation of our work with *Pediculus humanus* has placed us in possession of further information concerning the relation of this bacillus to its host.

Sections of lice from a stock which has been inbred for nearly five years show a high percentage infested with this parasite. It is probable that crowding together in boxes for so long a period has caused these lice to be more uniformly infected than wild ones. In male lice the cocco-bacilli are seen in the folds of the *vesica penis*, whereas in females they occur in the vaginal orifice and passage leading to the ovaries¹. No evidence that the gut is involved has been obtained from the very numerous sections examined.

Preliminary culture experiments with the guts of 24 lice taken at random from the stock were carried out. The insects were washed in 2 per cent. lysol followed by rinsing in sterile salt solution and the alimentary tract was dissected out on flamed slides with sterilised needles. Each specimen was transferred by means of a platinum loop to a separate nutrient agar tube and placed at the lower part of the slope.

Half the tubes were placed under anaerobic conditions, six at 35° C., and six at 26·5° C.; the remaining twelve were incubated aerobically at similar temperatures. Growth took place in seven of the twelve kept at the lower temperature, including both aerobic and anaerobic tubes. Subsequently five of the remaining tubes, which had been transferred to the cooler incubator, also produced growths. All the cultures appeared similar both to the naked eye and on microscopic examination. Subcultures were plated from the tubes and found to be pure. Both subcultures and plates were made with ordinary

¹ See Nuttall (1917), *Parasitology*, ix. 293 for description of copulatory apparatus of ♂ and ♀.

nutrient agar and were kept aerobically at 35° C. Later attempts to cultivate direct from the dissected guts at 35° C. have proved successful, so that the failures at this temperature in the first trial must be attributed to some chance circumstance. It may be noted here that to dissect out the alimentary system of the louse without contaminating it with organisms infesting the copulatory apparatus would be very difficult if not impossible, as the pressure of the dissecting needles would tend to release the valves which close the openings to both the male and female organs. The later series of trials was therefore planned in order to decide whether the gut was infected or not.

Six males, six females, and a like number of nymphs (in which the copulatory apparatus is not yet developed), were taken from the stock and treated in the same manner as in the previous experiment except that the two terminal segments were left adhering to the gut.

This experiment resulted as follows: six of the tubes containing the female and five of those containing the male guts produced growths of similar appearance to those in the previous experiment. Microscopically, when stained by Gram's method, they all showed similar cocco-bacilli, apparently in pure culture. Subcultures on plates from two tubes selected at random from among the eleven infected supported this assumption. As regards the tubes containing the dissections of the guts of nymphs only one showed a growth, and this was obviously contaminated as the growth started apart from the introduced gut, was of an entirely different appearance and was composed of Gram-positive bacteria.

Cultivation therefore strongly supports the microscopic evidence obtained from sections. We conclude that this bacterium may be correctly termed a parasite of the copulatory apparatus of *Pediculus humanus* and we propose for it the name of *Bacillus pediculi*.

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- ARKWRIGHT, BACOT and DUNCAN (15. iv. 1919). The Association of *Rickettsia* with Trench Fever. *Journ. of Hygiene*, XVIII. 76-94.

AN HEREDITARY *RICKETTSIA*-LIKE PARASITE OF THE BED BUG (*CIMEX LECTULARIUS*).

BY J. A. ARKWRIGHT, E. E. ATKIN AND A. BACOT.

(From the Lister Institute of Preventive Medicine.)

(With Plate II and 1 Text-figure.)

THE KNOWN SPECIES OF *RICKETTSIA*.

THE forms under consideration resemble *Rickettsia prowazeki*—the supposed cause of typhus fever—which occurs in lice that have fed on typhus fever patients.

It may therefore not be amiss to describe briefly the organisms which have been grouped by various workers in the same category as *Rickettsia*.

The general characters of “*Rickettsia*” may be summarised as follows:

(a) Bodies of minute size, usually 0.5μ in diameter or less, of round or diplococcal shape, though very minute bacillary and even thread-like forms occur.

(b) Though resembling very small bacteria in general appearance, they stain much less readily than ordinary bacteria but can be coloured with Giemsa’s stain; they are readily decolourised by Gram’s method.

(c) Absence of motility.

(d) Resistance so far, with one exception, to attempts made to cultivate them on artificial media *in vitro*.

(e) Their occurrence in very large numbers in the gut and in some cases in other organs of blood-sucking insects.

The known organisms apparently belonging to this group are:

(1) The organism found in the tick—*Dermacentor venustus*—the invertebrate host of the parasite causing Rocky Mountain spotted fever.

This was first described by Ricketts and has since been very thoroughly studied by Wolbach (1919) and called by him *Dermacentroxenus rickettsi*.

It is found in very large numbers in the alimentary canal, salivary glands, muscles and other organs of the tick and is passed on to the next generation of ticks. Similar forms have been described in the blood of men and other animals infected with this disease; Wolbach records its regular occurrence in the tissues of the mammalian host. This organism varies considerably and according to Wolbach, has three distinct morphological forms.

(2) *R. prowazeki* described in detail by da Rocha-Lima (1916) and previously by Ricketts (1909) and Sergeant, Foley and Vialatte (1914). This is found in

masses inside the cells of the mid-gut of *Pediculus humanus* after the latter has fed on typhus patients. Although often present in large numbers free in the gut, it appears to multiply inside the epithelial cells. Wolbach and Todd (1920) have described similar forms in the tissues of human sufferers from typhus. The organism varies considerably in shape and size; usually it is round, diplococcal or oval, but often resembles short bacilli; thread forms also occur.

(3) *R. quintana* or *wolhynica*, first described by Toepfer (1916) as the cause of trench fever, occurs in enormous numbers in the lumen of the gut of lice (*P. humanus*) which have fed on trench fever patients. It is more constantly rounded, oval or diplococcal than *R. prowazeki* and is not known to have occurred in thread forms. This species also stains a deeper purple by Giemsa's method. Most writers agree that the organism does not occur inside cells. It may be identical with *R. pediculi*.

(4) *R. pediculi*, first described by Munk and da Rocha-Lima (1917) as an occasional inhabitant of the gut of normal (uninfected) lice and subsequently said by them to be present in trench fever lice. They say that it is indistinguishable from *R. quintana*. It seems probable that the supposedly normal lice in which the parasite has been found had fed upon convalescents from trench fever whose disease had not been diagnosed.

(5) A species of *Rickettsia* found in lice which had fed on persons suffering from "war nephritis" was described by Toepfer (1917), who believed that he could distinguish it from *R. prowazeki* and *R. quintana* by its morphology. This has not been confirmed.

(6) A form of *Rickettsia* which Munk and da Rocha-Lima (*loc. cit.*) found occasionally in a few batches of lice which had either fed on normal persons or on trench fever patients. Munk and da Rocha-Lima stated that the organism was larger and stained more deeply than *R. prowazeki*, that it occurred not only in the lumen of the gut but also inside the cells lining the alimentary canal, in contrast to *R. pediculi* and *R. quintana*. These writers also stated that the organism damaged the cells of the gut and interfered with the powers of the insect to digest blood; they believe it to be a special parasite of the insect and not to be associated with human disease. If Munk and da Rocha-Lima are correct in this opinion their organism resembles the next species and that found in *A. lectularius*, which is described in this paper, so far as restriction to the invertebrate host is concerned.

(7) *R. melophagi*. This organism has been described by Noeller (1917), Sikora (1918), Jungmann (1918), and others, who say it is constantly present in the middle part of the stomach of *Melophagus ovinus* (the sheep "ked"), in large numbers in the older adults, in smaller numbers in young adults, and even occurs in pupae or in very young adults which have not yet sucked blood. The parasite is, they believe, hereditary in the "ked" and not derived from the sheep. The forms described are slightly larger than those of *R. pediculi* or *R. prowazeki*, round, oval or diplococcal in shape. Threads have not been

observed and this organism lies on the surface of the epithelial cells. It has been cultivated on blood agar by Noeller and Jungmann.

(8) *R. ctenocephali* was found by Sikora (*loc. cit.*) in 20 out of 100 cat fleas examined. It is said to be very like *R. quintana*.

(9) The last-named writer found similar forms in smears from the Malpighian tubes of a mouse flea.

RICKETTSIA IN C. LECTULARIUS.

Our knowledge of the form we are about to describe, which has hitherto apparently escaped attention, resulted from an attempt to infect bugs with the virus of trench fever by feeding them on patients suffering from this disease. Examinations of smears made from the guts of these insects showed in nearly every case thread-like "bacteria" with some admixture of shorter, rod-like forms (Plate II, fig. 2).

Stained with Giemsa's stain there appeared to be an outer sheath which took the eosin rather lightly while in the interior were granules or groups of granules which stained more deeply and of a purplish hue.

In some cases these smears also showed numbers of small deeply staining coccal or diplococcal bodies which, although slightly larger than *Rickettsia* found in gut and excreta smears of lice that had been fed on trench fever patients, were still sufficiently like them in size and general appearance to suggest that they might be the same species modified by development in the body of an unusual host. The fact that these bodies were not detected in any of the earlier smears from control bugs, although the thread-like forms above described were present, led to unsuccessful attempts to infect two volunteers with the emulsified guts of infected bugs in which the *Rickettsia*-like bodies were present. The examination of further control smears showed, however, that these minute bodies were also present in bugs that had fed only on normal men.

Suspicion of a relation between the rod and thread forms and these minute bodies arose owing to the occurrence of darkly stained granules in the rod and thread forms.

That these minute bodies frequently escaped our notice in smears which showed the long bacterial forms, is due partly to their small size, which makes it difficult to recognise them unless a considerable number are present in a single field; the same difficulty has been found in the case of the *Rickettsia* of trench fever. The fact that their distribution is often very localised is, however, the chief cause of difficulty in their detection. Whereas the bacillary forms are nearly always generally distributed in smaller or larger numbers in smears of well teased guts or Malpighian tubules, the minute forms, even when present in large numbers, are frequently only to be found in proximity to fragments of the gut or tubules. In some smears they were only seen where they chanced to be escaping from the ruptured end of the gut or a

tubule and no doubt the stage of the parasite in the infected cells affects the readiness with which it is found.

MOTILITY.

A study of fresh wet preparations by either transmitted light or dark ground illumination failed to reveal any signs of motility.

STAINING.

The most effective staining process tried is the slow method with weak Giemsa stain as generally employed for Rickettsia work, viz. 1 drop of stain to 1 c.c. of distilled water applied for 10–24 hours. All the forms are decolourised by Gram's method; the rod and thread forms are only faintly stained by the fuchsin counter stain. Strong fuchsin produces only a slight effect on the long forms and does not satisfactorily stain the minute coccal or diplococcal bodies. With Giemsa's stain the parasite does not react uniformly, the character of the tissues in its immediate vicinity apparently exercises an influence on the staining process. Where infected cells have been ruptured immediately prior to fixation or if the parasite is included in unbroken cells the long forms stain more intensely than when they are free. In the former situation the stained organism is purple, or if red, shows a gradation to the purple of the internal granules instead of an abrupt transition. It would appear that once the bacillary forms are removed from their natural habitat they rapidly undergo some change which causes them to stain badly.

CULTIVATION.

Attempts were made to cultivate the organism on artificial media. All the ordinary media were tried, aerobically and anaerobically, and in addition Dorset's egg medium, Noguchi's medium for spirochaetes, Krumwiede and Pratt's (1913) semi-solid medium which is successful in the cultivation of *B. fusiformis*, and the body juices of a lepidopterous pupa (*Hadena oleracea*), but in each case without a positive result.

In view of the remote possibility of the organism being a stage in the developmental cycle of a spirochaete, some mice were inoculated by scarification and subcutaneous injection with infected Malpighian tubes of the bug but no spirochaetes appeared in the blood.

EVIDENCE OF THE HEREDITARY CHARACTER OF THE PARASITE.

In the absence of evidence that the parasite had any second host, theoretical considerations based on the feeding habits of *Cimex lectularius* suggested that the organism must be passed on through the egg. An examination of smears made from the alimentary system of newly hatched unfed bugs showed that the latter were infected. Eggs washed in 2 per cent. lysol for five minutes and then in sterile salt solution contained both rod and thread forms, the

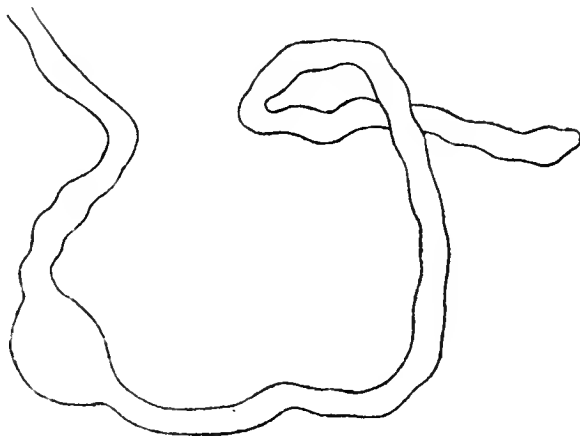
former being more common. This was also the case in smears of unhatched bugs extracted from the egg. Smears of eggs showing the early developmental stage of the bug (as a rule these eggs have to be dissected out of the ovary or made into smears soon after laying), contain immense numbers of more or less well-stained granules, usually poorly defined, but in some cases of distinct *Rickettsia* form and sharp in outline (Plate II, fig. 7); this feature may characterise the greater part of the smear. More frequently, however, it is confined to small patches in what are otherwise areas of ill-defined granules. Since we failed to cultivate the organism we cannot tell whether the ill-defined granules are related to the parasite or whether they are merely particles of protein. The sharply outlined forms are, we believe, undoubtedly a stage in the life-cycle of the organism. The minute bacillary forms that are present among the coccal and diplococcal forms are much more numerous in some smears than in others. Smears of the guts of embryo bugs at a late stage of development extracted from the egg, in some cases show masses of the minute forms deeply stained and clearly defined, lying within the lumen of the gut, where they have presumably been isolated as a part of the detritus of the embryonic process.

In order to obtain additional proof of hereditary infection, ovaries were dissected out of females that had been washed in lysol and then in sterile salt solution. Smears made from eggs extracted from these ovaries usually showed a few threads with some admixture of the shorter bacillary forms in addition to the granular infection alluded to above.

INTRACELLULAR MULTIPLICATION AND DEVELOPMENT.

Apart from its presence in the undifferentiated egg mass, the development of the parasite seems to be entirely intracellular. No evidence has been obtained of its multiplication in the lumen of the gut or in the body cavity where, however, thread forms have been found. Smears of teased embryos taken from developing eggs and of various organs from older bugs including the ovaries, testes, organ of Berlese and Malpighian tubes show minute coccal, diplococcal and lanceolate forms. These latter are slightly larger than the coccal and diplococcal (*Rickettsia*) forms and stain red instead of purple. Clusters of these red staining forms (Plate II, fig. 1), sometimes accompanied by a few of the *Rickettsia* forms, occur in the cytoplasm. They are best observed, individually, when the infected cell has been ruptured in such a manner as to spread its contents without breaking its nucleus. Very many examples of intermediate forms between these bodies and the longer bacterial forms have been observed; some clusters showed the lanceolate forms together with their various stages of growth into rods (Plate II, fig. 3). The intermediate forms were very often curved. Growth of the rods into threads is shown in almost every smear but apparently only in the enlarged cells of the Malpighian tubes do the threads attain their full length (Plate II, fig. 8).

The minute forms are also to be seen in some of the cells of teased guts in small numbers. In the cells of the seminal pocket on the ventral side of the fourth abdominal segment of adult females, known as the organ of Berlese¹, and in the cells of the Malpighian tubes clusters of the lanceolate forms are of frequent occurrence, sometimes very large ones (Plate II, fig. 4), while the *Rickettsia* forms are frequently present in immense numbers in the enlarged cells of the tubes in company with the thread forms. There would seem, however, to be a distinction between the course of intracellular development in the two organs in that, so far as present observation goes, threads and rods are comparatively scarce in the organ of Berlese, whereas they are exceedingly plentiful in the infected Malpighian tubes, generally in excess of the *Rickettsia* bodies. The longer bacterial forms are often present in the cells of certain portions of the gut wall, but they are never very numerous in any one cell.



Text-figure. Swelling in Malpighian tube of *Cimex lectularius* due to infection by the parasite.
Camera lucida drawing from unmounted dissection of adult female.

Within the cells of the Malpighian tubes the multiplication of the parasites becomes so great that individual cells are swollen to more than twice their normal diameter. This has only been observed in the larger nymphs or adult bugs. It is not uncommon to see two or more tubes each with one or more swellings in the same insect and as the tubes at these points may be two or even three times their normal diameter (Text-figure) the infection is quite apparent under the dissecting microscope.

The enlarged cells in fresh preparations are more transparent than normal ones, sometimes showing a faint greenish hue in the centre of the swelling, and when opened they are found to be packed with the thread forms and granules in varying proportions (Plate II, fig. 8). In sections such cells have a bird's nest appearance owing to the intertwining of the long bacterial

¹ The process of fertilization in the bed bug differs very remarkably from that usual among insects. The penis is inserted into an opening in the right side of the ventral plate of the fourth abdominal segment of the female. Immediately above this opening lies a spherical organ consisting of a mass of cells known as the organ of Berlese. This organ has no duct or outlet and the spermatozoa penetrate through the mass of cells composing the organ into the body cavity, finding their way to the oviducts and fertilizing the eggs *in situ*. The development of the embryo is frequently well advanced before the egg is laid. (For further details see Craig (1915), *Indian Journal of Medical Research*, II. No. 3, pp. 698-705.)

forms (Plate II, fig. 6). Presumably such cells eventually rupture and discharge their contents into either the body cavity or the lumen of the tubule, but this process is not shown in any section yet examined.

RELATION OF THE *RICKETTSIA* BODIES TO THE THREAD FORMS.

Under dark ground illumination the thread forms have been observed to evacuate granules which, judging by their size, are similar to the darkly stained bodies seen within the thread forms in smear preparations.

Circumstantial evidence suggests that these granules are the same as the *Rickettsia* bodies which in the first instance attracted our attention and that in bugs which have fed these bodies are largely derived from disruption of thread forms.

PROBLEM OF THE INFECTION OF THE EGGS.

In addition to their development in certain cells of the alimentary system, thread forms have been found in the blood of the bug taken with a fine capillary pipette from the cut stump of one of the legs of a female and heavy infection of certain cells of the organ of Berlese has been observed in an apparently virgin female (no spermatozoa were observed either in this organ or in a smear of the ovaries which contained no developed eggs). The smear of the ovaries of this female also showed infection, but it was uncertain whether the egg cells were themselves infected. In the case of this apparently virgin female the form found in the sexual organs was chiefly the lanceolate form.

The testes of the males are also infected in some cases but no signs of the parasite were found in smears of the accessory glands of the same insects. As already stated there is no evidence that the organism is motile. It seems possible that the eggs may be infected by the spermatozoa but attempts to obtain evidence from teased preparations that the spermatozoa were infected gave only negative results.

GENERAL CHARACTER OF THE INFECTION.

Heavy infection with this parasite must be very general, if not universal, among bed bugs. The stock of bugs in which the parasite was first noticed originated from a few specimens taken from an old Essex cottage and as the insects had been confined in a small box and inbred for many generations prior to their use for the trench fever experiments above-mentioned, it is not surprising that almost every bug examined showed one or other of the forms of the parasite. Another race was obtained from an old house situated in Paddington and smears were made from bugs of this stock before breeding from it. Insects of this stock were also found to be generally infected. A further supply was obtained from animal cages in a London laboratory and examined without giving them any chance of further feeding, 19 out of 20 showing the presence of the parasite.

Bugs from two different sources in Warsaw were examined within a day or two of capture and all these were found to be infected.

DEVELOPMENTAL CYCLE OF THE PARASITE.

The scheme set out below is merely tentative, all that is claimed for it is that it is a reasonable explanation of the observed facts and the excuse is offered that by linking up these facts a clearer picture can be conveyed than by the mere recital of the detached details. In the absence of any success in cultivation apart from the host, it is of course only possible to assume a connection between some of the forms in the order in which they are stated.

Starting with the eggs within the ovary, it seems most probable (*a*) that they become infected at the time of fertilisation with the *Rickettsia* form (Plate II, fig. 7), (*b*) that simple multiplication is followed by some of the first generation developing through a bacillary stage into threads while others continue simple multiplication *pari passu* with the presentation of suitable conditions due to the development of the embryo, (*c*) that owing to the massive granule infection of the egg material at this stage, cells of practically every organ of the growing embryo tend to become involved, but only in a certain number are the conditions necessary for intracellular multiplication afforded. In these, clusters of the minute *Rickettsia* forms develop, rapidly changing in favourable situations into the red-staining lanceolate forms through the development of an outer covering or envelope. These enlarge in due course into the long bacillary forms which in their turn release the darkly staining minute *Rickettsia* bodies. The large cells of the Malpighian tubules and their free unencumbered position allow of a much more massive infection than other situations; it is also possible that they afford more stable conditions during the moulting periods and bring the organisms into close proximity to the sexual glands when these are developed, thus increasing the chances of the latter becoming infected. In the course of the very numerous dissections which it has been necessary to make in the progress of this work, it has been noticed how frequently the enlarged cells occur in the distal third of the tubules.

SUGGESTED NAME FOR THE PARASITE.

The organisms known as *Rickettsia* are at present very incompletely described and only recognisable by their more superficial characters, such as morphology and localisation.

On such evidence it is not possible to decide with any certainty how near or remote the phylogenetic relationship of the members of the group to one another may really be, and the present writers therefore consider that *Rickettsia* should not be accepted as a generic name in the strict sense of the term to cover all the organisms referred to in their opening paragraphs. The smaller group comprising only those concerned with the three mammalian (human) diseases typhus, trench, and Rocky Mountain spotted fevers, would seem to form a more convenient unit.

Nevertheless, since the Rocky Mountain spotted fever organism has been

put into a new genus by Wolbach and on the other hand the form in the sheep "ked" which is not believed, however, to infect the sheep has been called *Rickettsia melophagi* it will be useful to retain *Rickettsia* as a group name for all in the present state of our knowledge. It is in this latter sense that we use it in tentatively naming the above-described parasite of the bed-bug *Rickettsia lectularia*.

POSTSCRIPT.

A recent examination of the few specimens of *Cimex hirundinis* which comprise all the bugs of the genus *Cimex*, apart from *C. lectularius* we have been able to obtain up to the present, shows that this species is also parasitised in some of the same organs which are infected in *C. lectularius*. As both the ovaries and testes are involved it is very probable that this will also prove to be an hereditary infection. The organism is, however, much more bacillary in appearance, considerably larger in size and has different staining reactions; in spite of several similarities it is not possible in the present limited state of our knowledge to decide how nearly, if at all, it may be related to the organism described above. We hope to obtain further material to continue our research and should any of our readers be able to obtain living specimens of any of the species within the genus *Cimex*, other than *lectularius*, we shall be very grateful to them if they will send us some.

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DESCRIPTION OF PLATE II.

- Fig. 1. Minute lanceolate forms issuing from ruptured cell of Malpighian tube ($\times 1000$).
Fig. 2. Bacterial forms in fragment of gut wall ($\times 1300$).
Fig. 3. A large cluster of lanceolate forms and developing bacterial forms issuing from ruptured cells of ovary ($\times 1000$).
Fig. 4. Minute lanceolate forms issuing from ruptured cell of organ of Berlese ($\times 1300$).
Fig. 5. Thread forms issuing from ruptured end of Malpighian tube ($\times 1000$).
Fig. 6. Section through Malpighian tube showing mass of thread forms in infected cell ($\times 1000$).
Fig. 7. Rickettsia and bacillary forms from smears of two eggs. The Rickettsia forms are more heavily stained than is usual in smears of eggs ($\times 1300$).
Fig. 8. Smear of Malpighian tube showing Rickettsia and thread forms ($\times 1300$).
Fig. 9. A range of forms selected from smears of various organs ($\times 1300$).

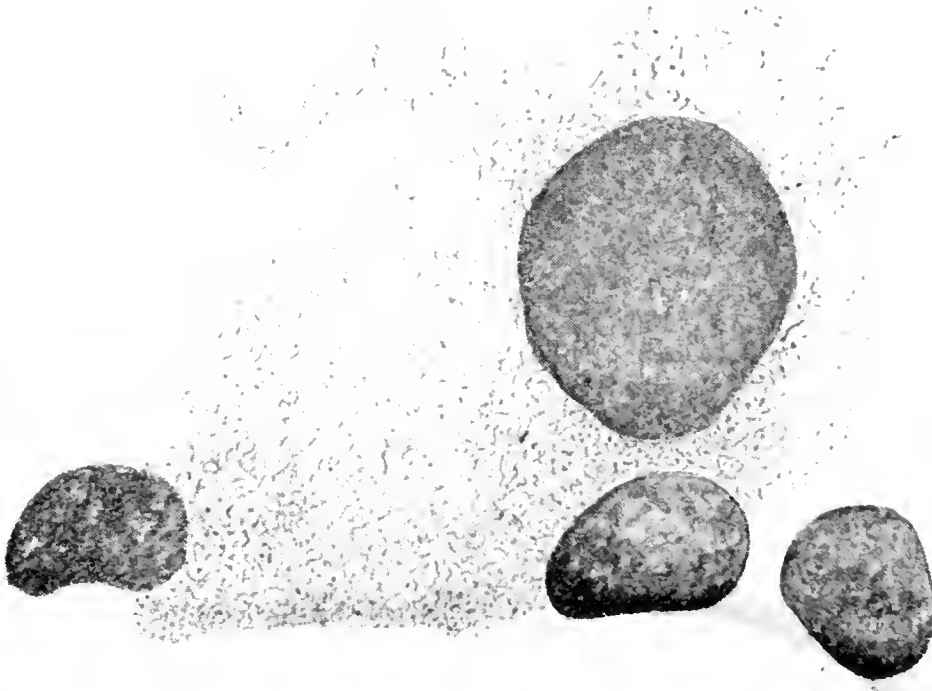




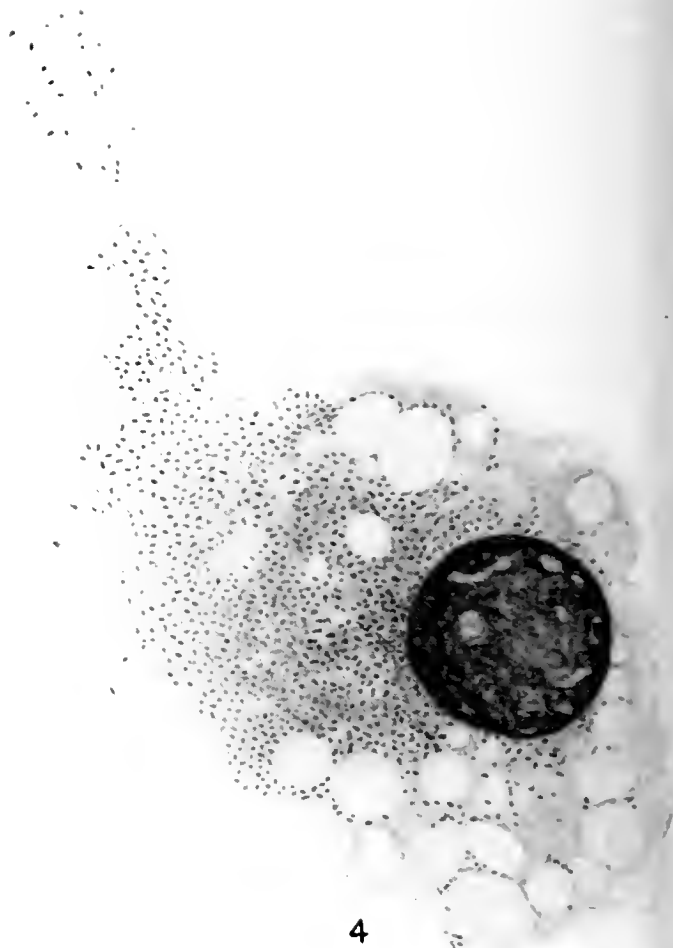
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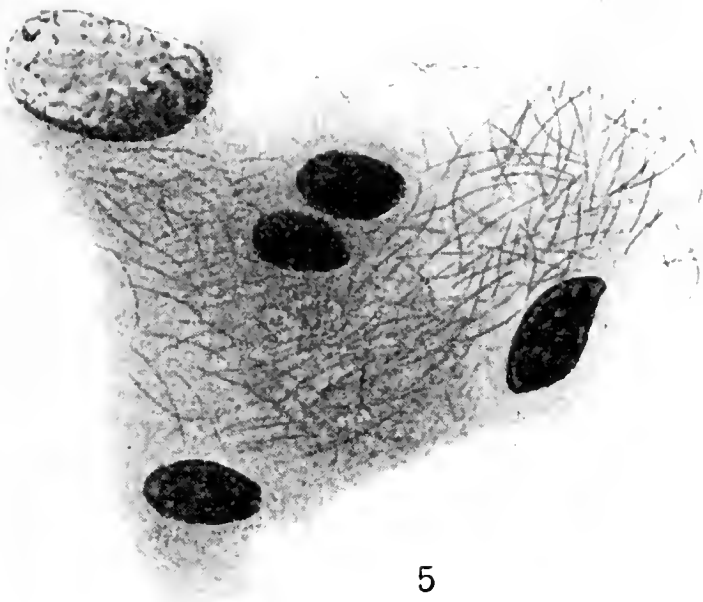
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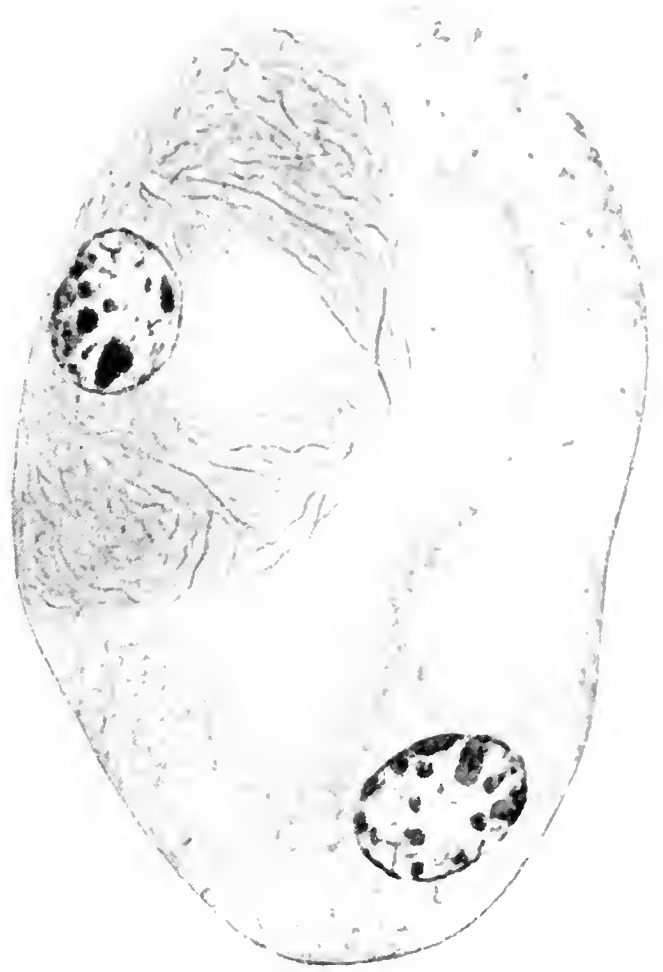
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ON THE LIFE HISTORY OF *ASCARIS LUMBRICOIDES*, L. PART V.

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(From the Lister Institute of Preventive Medicine, London, and the Quick Laboratory, Cambridge.)

(With 8 Text-figs.)

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I. SUMMARY OF THE LITERATURE ON THE SUBJECT PUBLISHED DURING 1919 AND 1920.

SINCE the publication of Part IV (Stewart, 1919), four papers dealing with the life-history of *Ascaris lumbricoides* have appeared.

(1) Ransom and Foster (1920) give a full account of their work, preliminary reports of which have already been published (1917 and 1919).

As evidence in the problem of whether the worm can undergo full development in one host alone, they attach great importance to two experiments, one on a kid and one on a lamb, in which after the administration of ripe eggs of *A. suilla*, they recovered worms from the intestines. In the kid, which died 24 days after the first feeding, the worms measured from 4·3 to 11·4 mm.; in the lamb, which was killed 103 days after feeding, the worms measured from 60 to 110 mm. The authors consider that, as *Ascaris* infection is uncommon in goats and sheep, the worms may without doubt be ascribed to the experimental feeding. They record five experiments on pigs which gave doubtful results, since the pigs fed with *Ascaris* eggs were found, after varying periods,

to be on the average less infected with *Ascaris* than pigs which were kept as controls, and had not been so fed.

They summarise their results as follows: "Stewart's observations as to the migration of *Ascaris* larvae through the lungs have been confirmed, but his suggestion that rats and mice act as intermediate hosts is not tenable. No intermediate host is necessary, and human beings and pigs become infected with *Ascaris* as a result of swallowing the eggs of the parasite, and not as the result of swallowing food, water or other substances that have been contaminated by the faeces of rats and mice." They base this view on the experiments referred to, and on the apparent death of larvae passed in the faeces of mice.

[I wish to emphasize that this view rests on the discovery which I was the first to make and to publish (Stewart, 1917 *a*), namely, that the larval development of the worm can take place in pigs as well as in rodents. Since making this observation I have asserted the possibility of direct infection. I maintained, however, that the mode of infection had not yet been conclusively demonstrated, that it was possible that the larvae passed in the faeces of mice were not actually dead, but in a condition of suspended animation, and that consequently indirect infection might also be possible.]

Ransom and Foster's paper is a very scholarly exposition of all work bearing on the subject. It contains sections on the relation of age to infestation which also exhibits the high frequency of the parasite among swine in the United States, on the egg stages of *Ascaris*, and on the life-history of related Nematodes. Of great theoretical interest is the observation that the larvae of *Ascaris anoura* apparently pass through the lungs of its host—a python—and reach a much higher stage of development there than do the larvae of *A. suilla* in the lungs of the pig.

In experiments on guinea-pigs the authors found *Ascarid* larvae in the lungs after the subcutaneous injection of ripe eggs of *A. suilla*. In feeding experiments they also found a small number of larvae in a few cases in the spleen and thyroid gland and under the peritoneum. They suggest the explanation that some young larvae may pass in the blood stream direct through the liver and lung, and thus entering the arterial system may be carried to situations remote from their normal route.

(2) Sadao Yoshida (1919) finds that hatching and larval migration of *A. lumbricoides* take place in guinea-pigs, monkeys and rabbits, and that human beings can be infected by larvae from the lungs of guinea-pigs. He has not yet completed the morphological study of the larvae.

(3) In a second paper, Yoshida (1919 *a*) develops the thesis that the larvae migrate through the serous sacs and direct through the tissues, not along the blood stream. This view he bases (1) on experiments in which he injected larvae extracted from the organs of one animal into the serous sacs of a second, and thereafter recovered them from remote tissues of the second animal. (2) In another series of experiments he administered ripe eggs by

the mouth, and found larvae not only in the liver and lungs, but in the abdominal cavity, spleen, pancreas, kidney, and pleural sacs.

He found larvae in the tissues at very short periods after infection, in one experiment in the pleura, lungs, abdominal cavity, liver, spleen, pancreas, and kidney after 20 hours only.

(4) I (Stewart, 1920) published a short summary of past work, and of some of the facts of distribution, and of the clinical importance of the parasite.

II. THE AUTHOR'S EXPERIMENTS DURING 1920.

The experiments dealt with in the present paper were conducted in the Lister Institute of Preventive Medicine during the summer of 1920, the cost of the animals used being borne by a grant from the Medical Research Council. The specimens secured were examined partly at the Lister Institute and partly at the Quick Laboratory, Cambridge. My thanks are due to the governing body of the Lister Institute, to the Medical Research Council, to Dr C. J. Martin and Dr MacConkey for much assistance, to Prof. Nuttall for his kind permission to work in the Quick Laboratory and for his help, and to Messrs C. and T. Harris of Calne for the supply of material.

The objects of the experiments were: (A) To ascertain whether after the administration of ripe eggs of *A. suilla* to pigs, the resulting worms could live and develop in the intestine after the 14th day. The experiments described in Part IV (Stewart, 1919), had traced the life-history to the 14th day, but I had failed to recover worms on the 19th day. (B) To observe whether larvae of *A. suilla*, passed in the faeces of mice, could infect pigs.

(A) *Experiments in which eggs of A. suilla were administered to four pigs.*

11. vi. 20. Ripe eggs of *A. suilla* from cultures 30–45 days' old, were given to four sucking pigs aged three days. Each pig received 20,000 to 30,000 eggs.

18. vi. 20. On the 7th day after infection all four pigs were seriously ill with pneumonic symptoms. Pig I died during the night, and larvae of *A. suilla* were found in enormous numbers in its trachea.

26. vi. 20. On the 15th day after infection Pig II was killed. *A. suilla* larvae, 3–5 mm. in length, were found throughout the small intestine, on the average one specimen in every centimetre of gut examined. The mucosa was in a catarrhal condition. Caecum and rectum negative.

28. vi. 20. On the 17th day after infection, Pig III was killed. *A. suilla* larvae, 4–7 mm. long, were found in its small intestine, one specimen in every 7 cm. of gut examined.

30. vi. 20. On the 19th day after infection, Pig IV was killed. *A. suilla* larvae, 6–7 mm. long, were found in its small intestine, one specimen in every 8 cm. of gut examined.

The foregoing experiments therefore resulted in the finding of larvae in the small intestine on the 15th, 17th and 19th days after infection. They had grown from 3 mm. on the 14th day to 7 mm. on the 19th.

(B) *Experiment in which A. suilla larvae in the faeces of mice were administered to two pigs.*

Ripe eggs of *A. suilla* were administered to eight mice. The faeces dropped by the mice on the 12th and 13th days after infection were collected, and found to contain immobile *Ascaris* larvae. After an interval of 14 days the faeces were administered to two sucking pigs three days old. The pigs were killed three days thereafter, and no worms were found in their intestines.

The experiment was therefore negative, but taking the technical difficulties into consideration, it would require to be repeated several times to establish with certainty that the larvae passed in the faeces of mice are non-infective.

III. THE ANATOMY OF THE LARVAE OF *A. SUILLA* DUJ. FOUND ON THE 19TH DAY AFTER HATCHING IN THE SMALL INTESTINE OF THE PIG.

Size. Length while alive 7 mm., when fixed in 70 per cent. alcohol 6.5 mm. Maximum breadth in alcohol 0.15 mm. *Head* (Fig. 1). 0.04 mm. long, lips connected at their bases by membrane, papillae as in the adult, no teeth, head separated from the body by a shallow circular groove. *Body.* Surface markings (1) annuli as in the adult, 0.005 mm. broad, (2) longitudinal ridges (Fig. 3) 30 to 32 in each (dorsal and ventral) half, ridges not continuous from one annulus to another nor in the same longitudinal line in successive annuli. Anus situate 0.2 mm. from the tip of the tail. *Tail.* As in the larva of the 14th day after hatching. *Cuticle* (Fig. 2). Consists of three layers, the mid-layer stains deeply with iron haematoxylin.

Lateral membranes (Figs. 2 and 4) extend from the neck nearly to the tip of the tail, are formed of all three layers of the cuticle, the mid-layer gives rise to the tri-radiate skeleton, the inner layer covers the lateral line and partly fills the triangular space underlying the skeleton with irregular lamellae. *Lateral lines* (Fig. 3) divided as in the adult by two very fine horizontal septa. The lateral canals are attached to their inner surfaces—*vide infra*.

Alimentary Canal. Mouth triangular in transverse section, funnel-shaped. Oesophagus 0.7 mm. long, simple club-shaped, slightly expanded also at anterior extremity; cuticular lining and outer membrane very fine; radial muscles well developed, embedded in a stroma of protoplasm, oval nuclei present in this stroma, number in front of nerve ring—in dorsal segment 5, in subventral segments 4 each; behind nerve ring—in dorsal segment 5, in left subventral 4, in right subventral 5; the posterior end of the dorsal segment contains the body of a large nucleus, which extends across the entire segment, and sends two branches forward along the upper edges of the subdorsal radii, and two branches downward and forward into the subventral segments.

Nerve ring. 0.182 mm. from the anterior extremity, contains one nucleus opposite each lateral line and each subventral muscle field.

Excretory system. Pore 0.238 mm. from the anterior extremity opposite the junction of the anterior and middle thirds of the oesophagus. The excretory duct is formed by the cells of the ventral line, and is lined by a fine membrane. The body of the excretory cell (Fig. 4) lies as usual beneath the oesophagus, joining the two lateral lines. The single large nucleus, 0.024 mm. long, lies in the left half of the cell. From the body two thin thread-like prolongations extend backward nearly to the level of the anus, intimately attached to the inner surfaces of the lateral lines (Fig. 3). Each prolongation contains a fine canal from its hind end to 0.5 mm. from its anterior extremity. It will thus be seen that these canals cannot be traced into the body of the cell or into connection with the excretory duct. The prolongations are the lateral canals.

Gonads. Male—a single solid rod of cells ventral to the intestine, 2 mm. long, extending forward from the anus. Female—vulva patent, in the ventral line, 3 mm. from the tip of the tail. The vagina extends backwards and slightly to the left, and divides into two utero-ovarian tubes, one lying ventral to the intestine, the other along its left side. The former reaches to 2 mm., the latter to 2.3 mm. from the tip of the tail.

IV. THE DEVELOPMENT OF CERTAIN ORGANS IN *A. SUILLA* DUJ. FROM THE EMBRYO AT HATCHING TO THE LARVA DESCRIBED IN SECTION III.

Cuticle and lateral membranes (Figs. 5, 6 and 7). In the embryo the cuticle is excessively fine, lateral membranes absent. The lateral membranes appear on the 5th day, and are recognisable both in larvae from the liver and from the lung: they are extremely small. They become more prominent, and the skeleton appears, during the passage up the bronchi and trachea on the 8th, 9th, and 10th days. The cuticle thickens markedly on the arrival of the larvae in the alimentary canal. The growth of the cuticle and lateral membranes appears to be continuous, and I have not observed any sign of an ecdysis¹.

Oesophagus. In the embryo it is represented by nuclei arranged as a narrow cylinder expanding at the hind end into a bulb. There is therefore no doubt as to the multicellular origin of the organ.

Excretory cell. The nucleus can be identified (Fig. 8) in the embryo on the left side, opposite the anterior end of the bulb. In sections of larvae of the fourth day after hatching, the cell can be seen to belong to the left lateral line. By the fifth day it has grown underneath the oesophagus across the mid-line, and established contact with the right lateral line. The lateral canals are formed between the 14th and 19th days after hatching. The excretory system, with the exception of the terminal portion of the duct, is composed of one very large branching cell only. The wall of the terminal portion of the duct is formed by cells of the ventral line.

¹ This statement refers only to larvae after entry into the tissues of the host. An ecdysis occurs immediately after hatching, in the alimentary canal of the host.

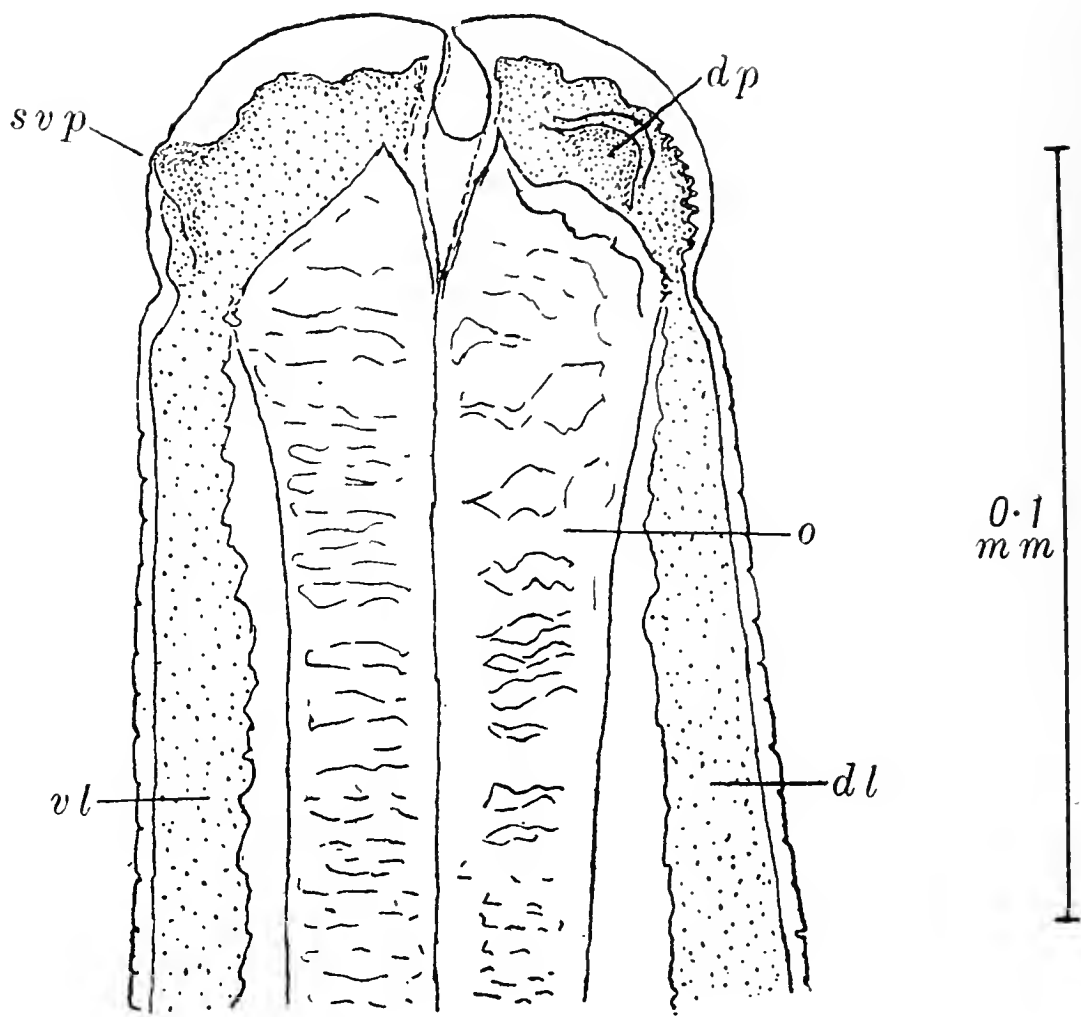


Fig. 1

0.01 mm

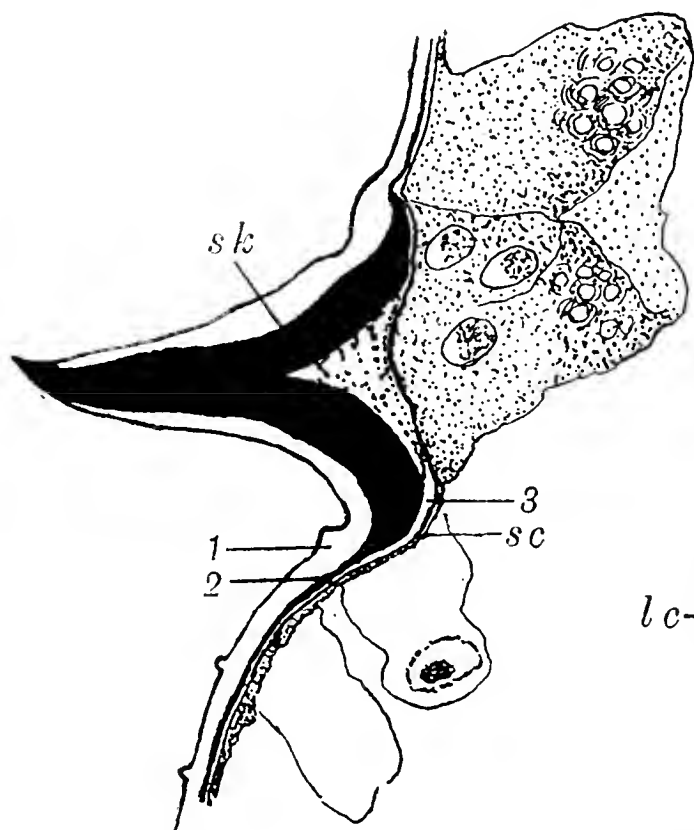


Fig. 2

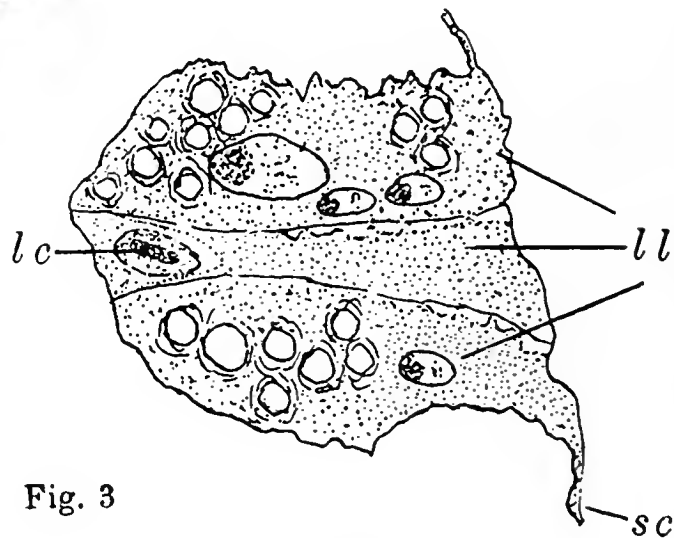
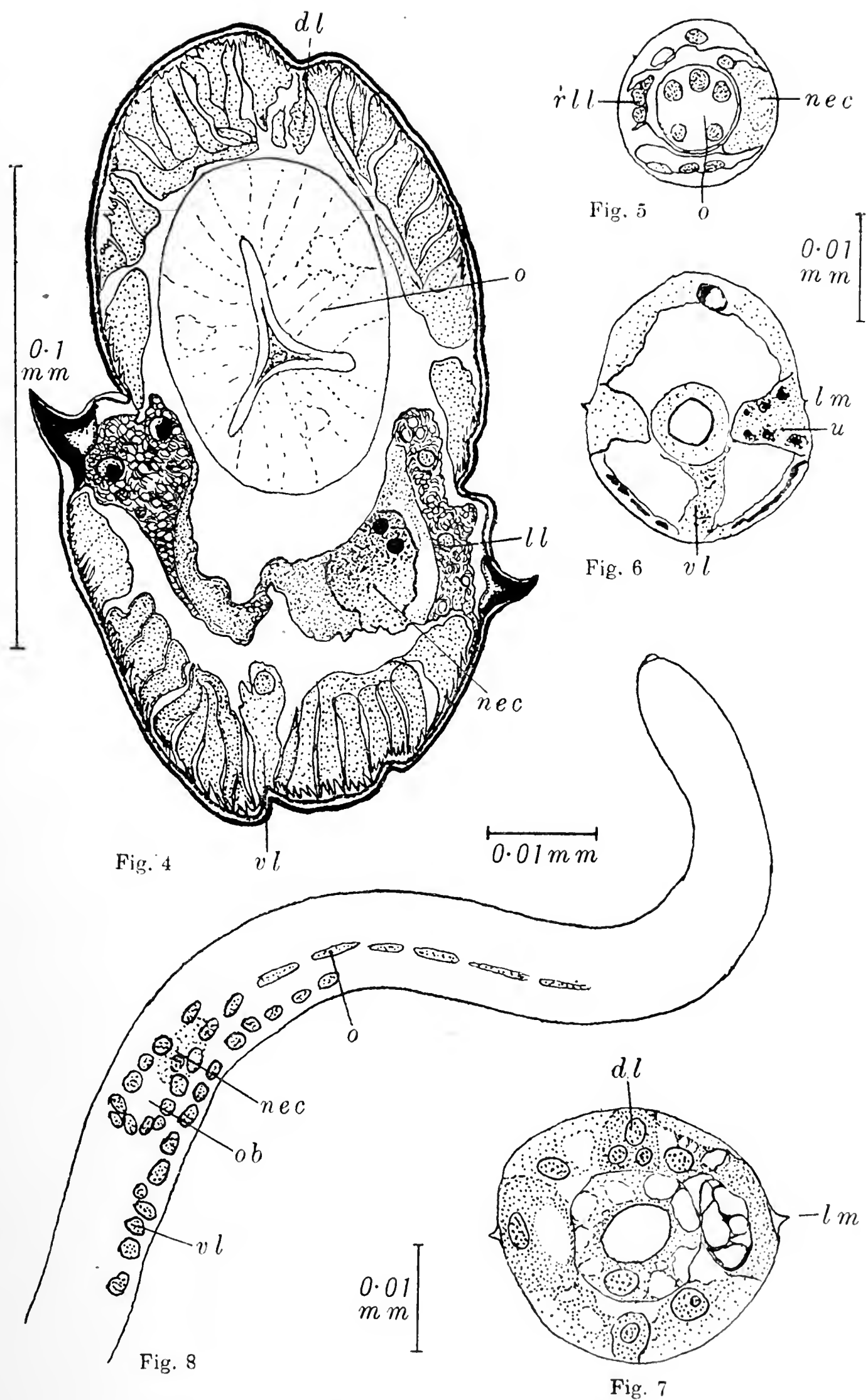


Fig. 3

0.01 mm

Figs. 1-3. *Ascaris suilla* Dujardin. (See Legends, p. 47.)



Figs. 4-8. *Ascaris suilla* Dujardin. (See Legends, p. 47.)

V. THE ROUTE TAKEN BY THE LARVAE OF *A. SUILLA* DUJ. IN THEIR
MIGRATION THROUGH THE TISSUES OF THE HOST.

The larvae of *Ascaris lumbricoides* and its relations live actually within the tissues of their hosts from the moment they perforate the walls of the intestine (assuming they do not ascend the bile ducts) until they reach the alveolar spaces of the lungs. Theoretically considered they could travel from the intestine to the lung along two paths, namely (1) by the veins and pulmonary artery, and (2) by the lymphatics and blood-vessels, and (3) by a third method directly through all intervening tissues, availing themselves only incidentally of such natural spaces as they encountered on the line which they happened to take.

The first is that which I suggested (Stewart, 1917, p. 226). It has been accepted with reservations by Ransom and Foster. It is the route followed by a minority of the larvae of *Agchylostoma*. The second is that followed by the majority of the Ankylostome larvae. Larvae coming from the small intestine would by this route traverse the lacteals, cisterna chyli, and thoracic duct to the left subclavian vein. The third route has been suggested recently by Yoshida. "It is believed that the larvae migrate in every direction boring through various organs or tissues by means of their own power of piercing but not by way of blood-vessels." "The general and important course of migration by the ascarid larvae in the body of the host may be as follows: The ascarid larvae proceed to the abdominal cavity by boring through the wall of the intestine. Thence they pierce the diaphragm to enter the pleural cavity, finally penetrating into the lungs from their surface. It might be considered as an additional and mere accidental course of migration that the larvae in the abdominal cavity penetrate into the liver, thence they are carried to the lungs by the way of blood-vessels, passing through the heart."

Yoshida advances the following arguments in favour of his hypothesis. (1) That he has found larvae in the abdominal cavity in large numbers during the first 48 hours, in lesser numbers thereafter. He has also found them in the pleural cavities and in organs lying off the direct blood route, viz. spleen, kidney, and pancreas. He also states that histological study shows "that almost all the larvae in the lungs and liver are not found in the blood vessels, but in other tissues." His infection experiments prove that the larvae can migrate from the serous cavities to the lungs.

The following considerations are opposed to this view: (1) In Yoshida's own experiments the liver was apparently found to contain as many larvae as the abdominal cavity during the first 48 hours. I have examined the pleural and abdominal cavities during the first 48 hours in three cases only, which of course do not permit of a conclusion, especially in view of Yoshida's very definite results, but I have failed to find larvae in these cavities, although they were present in the liver. (2) My experience of serial sections differs entirely from that of Yoshida. I have found that the majority of larvae in the

liver lie obviously in dilated blood capillaries, venules, and veins. In the latter situations they are surrounded on all sides by red blood corpuscles.

Ransom and Foster in one experiment found larvae in blood taken with a syringe from the portal vein, in another experiment one larva in blood from the pulmonary artery. They state, however, that accidental access of larvae from other sources could not be entirely excluded.

The following table shows the breadth of *Ascaris*, *Agchylostoma* and *Filaria* larvae, compared with the diameter of blood capillaries:

	Maximum breadth mm.
1. <i>Ascaris suilla</i> recently hatched, alive in salt solution ...	0.013
2. <i>Ascaris suilla</i> recently hatched, mounted in Canada balsam	0.0098
3. <i>Ascaris</i> larva of fifth day, from lung of rat, in weak sublimate solution	0.025
4. <i>Ascaris</i> larva of sixth day, from liver, in salt solution ...	0.034
5. <i>Agchylostoma duodenale</i> mature larva, living (Looss) ...	0.017
6. <i>Filaria bankrofti</i> larva from human blood, living (Manson)	0.008-0.011
7. Human capillary normally full of blood (Schäfer) ...	0.007-0.008

Comparing 1, 6 and 7, it is clear that an *Ascaris* larva within the first 24-48 hours could pass through a capillary with a moderate degree of dilatation, but that it would probably be delayed in the passage. Larvae of the fifth or sixth days would be much delayed, and might rupture the capillary.

The early appearance of larvae in the lungs, and in small numbers in remote organs such as the spleen and kidney, could be explained, as suggested by Ransom and Foster, by their passage direct in the blood stream through the liver, heart, and lungs. It is also possible that some traverse the lacteals, cysterna chyli, thoracic duct, subclavian veins, heart, and pulmonary artery, and thus reach the lungs without passing through the liver.

The marked degeneration and disintegration of the liver cells around the larvae would permit of slow abnormal dilatation of the capillaries by the older animals, and their passage onward into the hepatic venules and veins. Even if rupture of the capillaries occurs, the larvae regain the blood stream in the veins, as is seen in the sections referred to above.

Taking into consideration therefore the great frequency of the larvae in the liver, and their demonstrable presence in the blood vessels of this organ, I consider that the route described by me (Stewart, 1917, p. 226) is that followed by the great majority of the larvae, although a small proportion may doubtless follow the lymph stream, or perforate the diaphragm as described by Yoshida. In the blood and lymph streams they are guided by thigmotropism. It is not so easy to define the stimulus which would guide them under Yoshida's hypothesis, although we cannot believe that their movements are entirely anarchic.

VI. MECHANISM OF PROTECTION OF THE BODY AGAINST THE INVASION OF *ASCARIS* LARVAE.

Yoshida reports that larvae found in the pleural cavities are surrounded by groups of white blood corpuscles and histiocytes (Yoshida, 1919 *a*, p. 23). This is clearly the same phenomenon as the capture of the *Ankylostome* larvae in the lymph glands, two days after infection, as observed by Looss (1911, p. 521). This author showed that such larvae were killed by the lymphatic cells, and that this process forms part of the mechanism of defence. Strong infection stimulates the glands. "It seems as though the larvae were able to withstand the attacks of the lymphatic cells for a certain length of time or possibly also the lymphatic cells do not at once commence their attacks. If the larvae succeed in escaping from the glands within this time they are saved, if they do not succeed they fall a prey to the lymphatic cells." Captured larvae were observed in considerable numbers as early as two days after infection. In Yoshida's cases, the reaction occurred as early as 20 hours after feeding.

VII. THE EXTENT OF NATURAL *ASCARIS* INFESTATION OF PIGS IN ENGLAND AND TREATMENT SUGGESTED WITH A VIEW TO THE ERADICATION OF THE DISEASE.

Messrs C. and T. Harris & Co. of Calne, Wiltshire, at my request, very kindly undertook the enumeration of pigs found to contain *Ascaris* in their abattoirs. Out of 370 stores, 62 or 16.75 per cent. contained this worm, out of 29 sows, 3 or 10.34 per cent. were affected. These figures are very low compared with the American returns of Raffensperger, who found 41.1 per cent. affected (Ransom and Foster, 1920, p. 25). To estimate the economic effect of the parasite in this country, further statistics of infection from all districts are required, and also extensive observations on the relative weight of infected and uninfected pigs. The facts given by myself (Stewart, 1918 *a*, p. 203), although based on only 83 examinations, suggest the possibility of considerable loss from this cause.

Treatment. Since animals of two months and over appear to be only slightly susceptible to infection, it should be possible to free a herd completely of the parasite by a routine administration of santonin or oil of chenopodium, to all pigs at the ages of one and two months. Whether the adoption of such a routine on a large scale should be recommended or not, would depend on the results of the investigation suggested above.

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LEGENDS TO TEXT-FIGURES.

Figs. 1–8. *Ascaris suilla* Dujardin.

- Fig. 1. Larva 19 days after hatching, from the intestine of the pig. Head.
- Fig. 2. Larva 19 days after hatching. Transverse section through the lateral line and lateral membrane, 0.68 mm. from the anterior extremity.
- Fig. 3. Larva 19 days after hatching. Transverse section through the lateral line, 1.1 mm. from the anterior extremity.
- Fig. 4. Larva 19 days after hatching, from the intestine of the pig. Transverse section 0.35 mm. from the anterior extremity.
- Fig. 5. Larva 4 days after hatching, from the liver of the mouse. Transverse section through the oesophageal region.
- Fig. 6. Larva 5 days after hatching, from the liver of the rat. Transverse section through the intestinal region.
- Fig. 7. Larva 6 days after hatching, from the lung of the mouse. Transverse section through the intestinal region.
- Fig. 8. Embryo after incubation of the egg for 35 days, artificially hatched. Anterior portion of the body.

LETTERING.

e.c., excretory canal. *d.l.*, dorsal line. *d.p.*, dorsal papilla. *l.c.*, lateral canal. *l.l.*, lateral line. *l.m.*, lateral membrane. *n.e.c.*, nucleus of excretory cell. *o.*, oesophagus. *o.b.*, oesophageal bulb. *r.l.l.*, right lateral line. *s.c.*, subcuticle. *s.k.*, skeletal rod. *s.v.p.*, subventral papilla. *v.l.*, ventral line. 1, external layer of the cuticle. 2, midlayer of the cuticle. 3, internal layer of the cuticle.

A REVISION OF THE GENUS *FASCIOLA*.

WITH PARTICULAR REFERENCE TO *F. GIGANTICA* (COBBOLD)
AND *F. NYANZI* (LEIPER).

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(With Plate III and 4 Text-figs.)

THE material which is the subject of this paper was obtained from the following sources.

One tube of flukes collected by Dr H. H. Marshall at Bassein and Rangoon, British Burmah (referred to below as "Rangoon specimens")¹; two tubes sent by Mr H. E. Hornby at different times from Northern Rhodesia (referred to as "Rhodesia specimens," "1st collection" and "2nd collection" respectively); and two tubes (Nos. 29 and 65) from the Veterinary Pathology Laboratory, Nairobi, British East Africa, collected by Mr R. E. Montgomery (the former referred to as "Nairobi specimens")¹; in all comprising 40 species. I am very much indebted to Dr C. L. Boulenger, F.Z.S., Professor of Zoology at Lahore, for entrusting me with the description of this material.

The tubes contained elongated flukes of the genus *Fasciola*. It was found on examination that all the specimens (with the exception of those contained in the tube, Nairobi, No. 65) were related to *Fasciola gigantica* (Cobb.) and the question of the relationship of the various elongated flukes, that have been described from time to time under different specific names, with Cobbold's species and these new specimens, was thus raised again.

The question may be stated thus—do all these forms belong properly to *F. gigantica* (Cobb.) or should they be split up into more than one species? The history of the matter can briefly be told². *F. gigantica* was described by Cobbold (1856) from specimens found in a giraffe belonging to a travelling menagerie.—Railliet (1895) communicated the discovery of an elongated fluke in Senegal cattle, slaughtered at St Louis and provisionally named it *F. hepatica* var. *angusta*.—Looss (1896) described a fluke from cattle slaughtered at Cairo that he named *F. hepatica* var. *aegyptiaca*, but which he considered a distinct species in 1899.—Blanchard (1896) suggested that both these varieties were in reality the same as Cobbold's *F. gigantica* but he gave no evidence or figures to support this suggestion.—Looss (1902) returned to the subject after examining a large number of specimens from

¹ Received from the Quick Laboratory, Cambridge.

For detailed discussion of the earlier history and complete bibliography see Stiles 1894-95.

the Cairo abattoirs. He accepts Blanchard's suggestion and withdraws the species *aegyptiaca*, not entirely without misgivings¹, and further suggests that the *gigantica* type of fluke is an African form in the same way as *F. magna* may be looked upon as an American form. In this note he gives, however, no anatomical details and no figures.—Braun (1906), without adding any original observations, is willing to accept the identity of *F. angusta* and *F. aegyptiaca* with one another but is sceptical of their identity with *F. gigantea*.

The prevailing uncertainty on the subject is largely due to the imperfect description of *F. gigantea* given by Cobbold and the fact that it has not been added to or revised since his time. By the courtesy of Dr Arthur Keith, F.R.S., I was given facilities for examining the original specimens in the Royal College of Surgeons, and am able to record all that the state of preservation of the specimens will allow to be contributed towards the elucidation of this matter. The specimens are three in number and quite extraordinarily different. They will be described in order of size:

COBBOLD'S *FASCIOLA GIGANTICA*.

Specimen No. 1 (Text-fig. 1). 76 mm. long by 5 mm. at broadest part. Cone cylindroconical and mounted on sharply projecting "shoulders," the left in this specimen being more anteriorly placed than the right. The ventral sucker is large and prominent with an irregular triangular aperture. The greatest breadth of the body is attained just behind the right "shoulder" and it narrows so slightly that it has only decreased 1 mm. at the posterior end of the testis area—a length of 35 mm. from the mouth. The tail is bluntly pointed and the last 41 mm. of the body are entirely occupied by yolk glands and alimentary canal. Narrow strips of yolk glands continue laterally to the "shoulders." The testes occupy the usual position and are coarsely lobed. The shell gland is visible 12 mm. from the anterior end and the uterus is long and loosely coiled. The gut cannot be made out in detail and no ramifications of the lateral diverticula can be seen. Yolk glands appear to spread on both sides of the gut. Spines are not visible. Proportion of length to breadth, 15.2 to 1.

Specimen No. 2 (Text-fig. 2) is 59 mm. long and 7 mm. broad. The cone passes into the body insensibly, there being no distinct "shoulders." The sides are almost parallel, tapering ever so slightly towards the tail, which is bluntly rounded. The testes occupy more space than in No. 1 and are coarsely lobed. The remainder of the organs which can be made out are typical, or as figured by Cobbold. I make out, however, more than 8–10 branches of the gut diverticula and indeed Cobbold's statement conflicts with his figures in this respect. Size of eggs: length 0.145 mm. to 0.150 mm., breadth 0.082 mm. to 0.088 mm. Proportion of length to breadth over 8 to 1.

¹ "...Ich wüsste gegen die Berechtigung seines Vorgehens gegenwärtig nichts mehr einzuwenden, und ziehe *F. aegyptiaca* als selbständige Species zurück"

Specimen No. 3, 23 mm. long by 3.5 mm. broad, is a small fluke of similar proportions to No. 2, but the cone is larger in proportion to the body. The condition of the specimen makes it difficult to determine. The uterus is

large and with few coils; it contains some eggs. The space occupied by the testes is unusually large, from the mouth to the posterior border of the testis area measuring about 18 mm. The ventral sucker is not prominent. It is not possible to make out the alimentary canal. The yolk glands are normally arranged and the median commissures to the shell gland are visible. Proportion of length to breadth under 7 to 1.

Specimen No. 2 is a type of the specimen figured by Cobbold but somewhat longer in proportion to the breadth than his figure. The average length of his specimens is given as 2 inches and as this type specimen is nearly $2\frac{1}{2}$ inches it may be taken as slightly larger than the average. In the largest specimen (No. 1) the proportion of length to breadth is amazingly larger than any known fluke, and in the other specimens it is remarkably large. One can only look on the classing of these three worms in one species with grave suspicion until similar specimens are again found in the giraffe and their anatomy is accurately described.

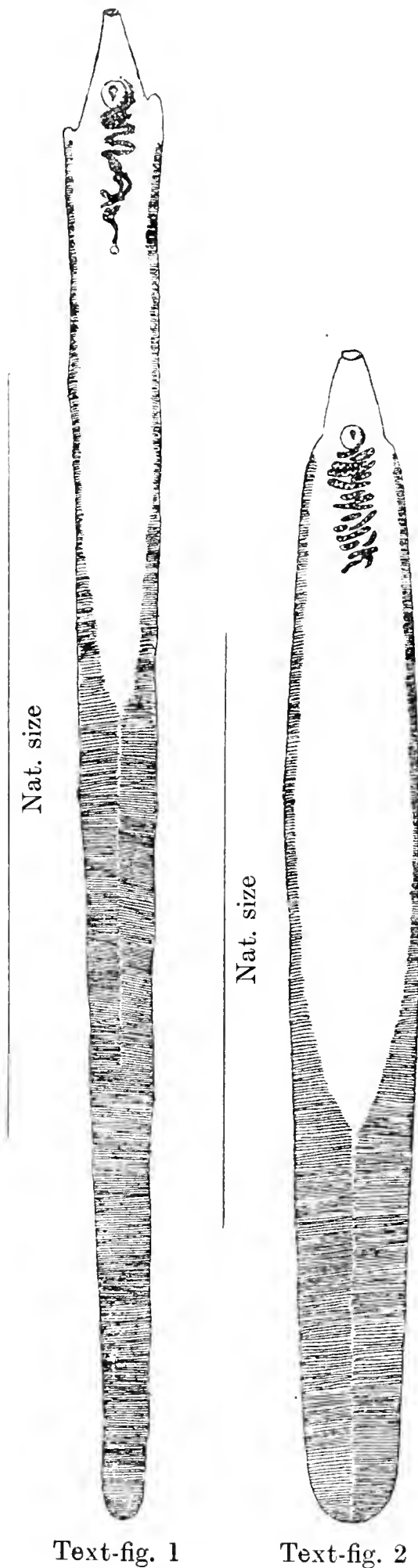
In the meantime the medium sized specimen (No. 2) must be taken as the type of *Fasciola gigantica*.

The material in my possession which bears on the specimens under consideration is described below, but only the important features are recorded.

RANGOON SPECIMENS (Pl. III, fig. 1).

Largest 45 mm. by 12 mm. Narrowest 8 mm. by 34 mm. Shortest 29 mm. by 9 mm.

The large cone passes insensibly into the body; in no case are "shoulders" more than slightly indicated. The body in every specimen tends to expand slowly to its greatest width at or about the posterior border of the testis area and ends in a very bluntly rounded tail. The ventral sucker is round and



Text-fig. 1

Text-fig. 2

prominent and its lumen is produced posteriorly into a triangular pouch. The pharynx is long and the oesophagus very short, the diverticula of the gut are very strongly branched externally and internally, and the internal branches are ramose. Yolk glands spread on both sides of the alimentary canal. The testis area is moderately large, its posterior margin being about one-third of the entire length from the tail. The ovary is on the right side and, as in all other points of the histology and finer anatomy, is similar to *F. hepatica*. The body is very flat dorsoventrally and covered with moderately large spines. The eggs are of sizes lying between 0.140 mm. to 0.160 mm. in length and 0.060 mm. to 0.100 mm. in breadth. Average proportion of length to breadth 3.2 to 1.

Locality. Cattle, Bassein and Rangoon.

RHODESIA SPECIMENS (Text-fig. 3). *1st Collection.*

Longest 39 mm. by 11 mm. Shortest and narrowest 36 mm. by 10 mm. Broadest 12 mm. by 37 mm.

These resemble the Rangoon specimens in general form, but the body reaches its greatest length nearer to the anterior end and tapers very gradually to a blunt tail. The ventral sucker has a triangular aperture and is slightly pouched posteriorly. The general anatomy is as above. The body is very flat dorsoventrally and the spines are moderately large.

Eggs: length 0.150 mm. to 0.170 mm., breadth 0.070 mm. to 0.085 mm. Average proportion of length to breadth 3.3 to 1.

Locality. Cattle, Northern Rhodesia.

RHODESIA SPECIMENS (Text-fig. 4) *2nd Collection.*

Longest 37 mm. by 9 mm. Widest 13 mm. by 26 mm. Shortest 25 mm. by 10 mm. Narrowest 8 mm. by 36 mm.

The majority of these specimens are more slender than any described above. The slenderness of the body gives the cone an appearance of large size—it is perhaps larger in proportion to the body than in the specimens already described. The “shoulders” are very slight. The body soon reaches its full width and retains it for one-third of its length, tapering posteriorly to a bluntly pointed tail. The anatomy is as above.

Eggs: length 0.130 mm. to 0.175 mm., breadth 0.075 mm. to 0.090 mm. Average proportion of length to breadth 3.7 to 1. In many specimens the proportion is over 4 to 1.

Locality. Cattle, Northern Rhodesia.

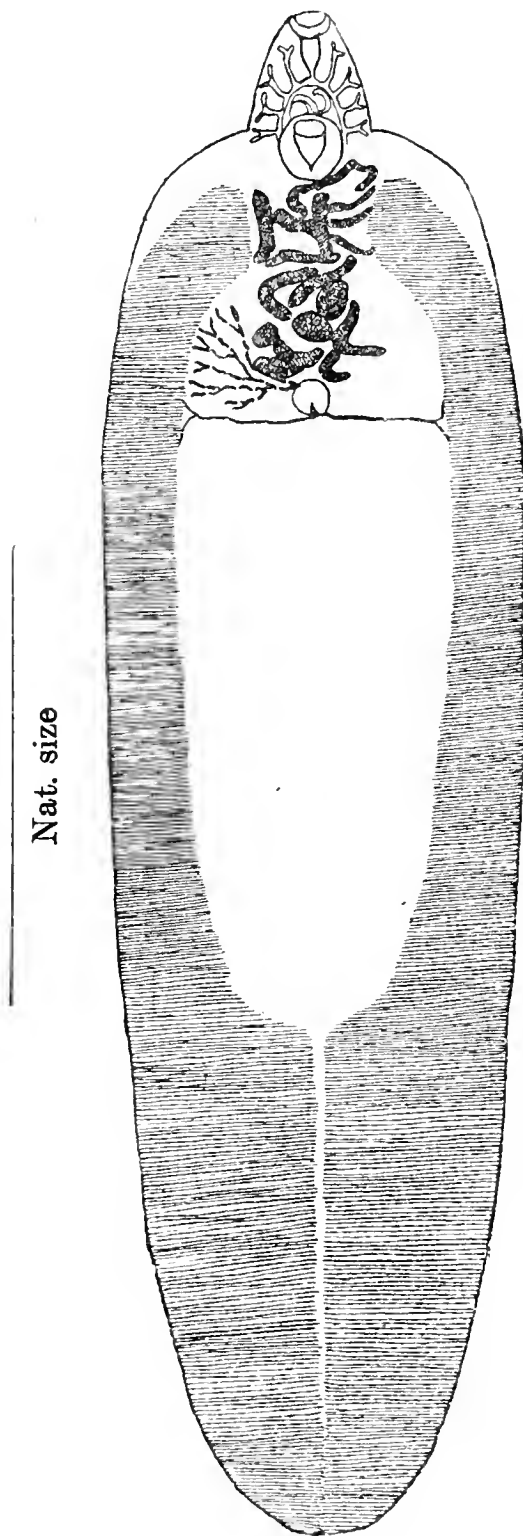
NAIROBI SPECIMENS (Pl. III, fig. 2).

Three specimens measuring 33 mm. by 7 mm., 35 mm. by 6 mm., and 34 mm. by 6 mm. and differing in slight points from those described above. The body is very slender and the large cone is, at its base, as wide as the body, no “shoulders” being present. It expands slightly for about one-third

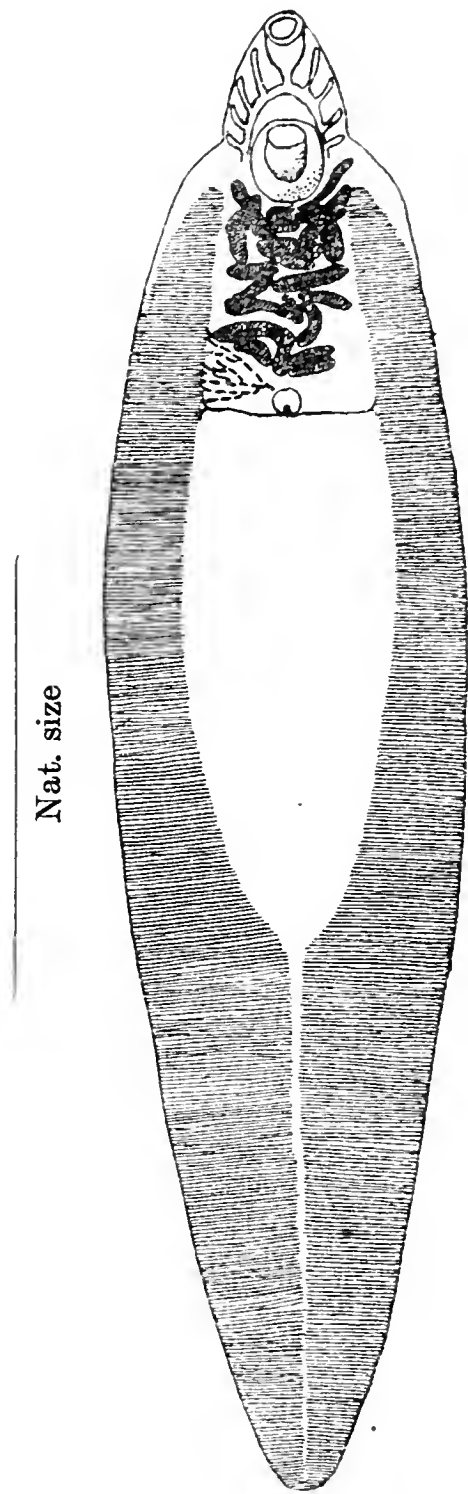
of its length and then tapers to a bluntly pointed tail. The ventral sucker is very large and protuberant and has a deep posterior pouch. The oesophagus is about half as long as the pharynx. Large spines cover the body, and smaller ones the cone. Average proportion of length to breadth 4·7 to 1. The anatomy is as above.

Eggs: length 0·125 mm. to 0·155 mm., breadth 0·075 mm. to 0·095 mm.

Locality. Ox, British East Africa.



Text-fig. 3



Text-fig. 4

DISCUSSION AND CONCLUSIONS.

The relationship of the flukes described above to those already described by Railliet and Looss admits of little doubt.

The Rangoon specimens can be seen at once to correspond in almost every detail with Looss' *F. aegyptiaca*; the only difference to be found is in the slightly narrower proportions, on the average, of Looss' specimens.

Similarly the Nairobi specimens can be referred to the variety originally described by Railliet under the name of *F. hepatica* var. *angusta*, from which they only differ in the slightest degree. The Rhodesian flukes possess no character which would separate them from either, the first collection coming nearer to the Rangoon specimens and the second collection to the Nairobi specimens.

The bridge between the two forms being thus complete, it may be taken that *F. angusta* and *F. aegyptiaca* are one and the same species.

Their relation to *F. gigantea* is less obvious. The great size of Cobbold's specimens may partly be accounted for by the undoubted maceration they had undergone before examination. Goddard (1919) records cases of extreme alterations in *Fasciolopsis*, measured immediately after evacuation and after standing a few hours in water. Shape and size cannot count for a great deal in the delimitation of species in these forms, owing to the distortion inevitably produced by maceration and fixation, unless coupled with distinct anatomical differences.

The anatomy provides us with little on which to base a separation. The alimentary canal is provided with the ramose internal branches of the diverticula, which have been shown to be characteristic of all the forms described above.

Cobbold's figures do not distinctly show the proportion of pharynx to oesophagus, but it is indistinctly indicated (it was not possible to make out the point in the original specimen I examined) that the oesophagus is quite as long as the pharynx. If this is correct—and the mobility of the muscular pharynx makes the character at least of doubtful value—it makes a point of difference from these recent specimens. The testes are similar and occupy a similar proportion of body space. The remaining organs are similar. The parasite comes from the same continent as all the elongated forms (except the Rangoon specimens) that have been hitherto described.

The eggs—length 0.145 mm. to 0.150 mm., breadth 0.082 mm. to 0.088 mm. lie within the limits of those of the specimens described above.

On the whole therefore the differences amount to so little that we may, without much hesitation, refer all these forms to the species *Fasciola gigantea* (Cobbold).

The descriptions given above may now be combined to make the following diagnosis of *F. gigantea*.

F. GIGANTICA (COBB.).

Body elongated and at least three times as long as broad. Cone passing almost insensibly into the body, prominent "shoulders" always being absent. As a rule the sides of the body are roughly parallel and the posterior extremity is bluntly rounded or more rarely bluntly pointed. The ventral sucker is large, often protuberant and its cavity is usually prolonged backwards into a blind pouch. The pharynx is longer than the oesophagus. The external branches of

the diverticula of the alimentary canal are as in *F. hepatica*, the internal branches, however, are numerous and always ramose to a greater or less extent. The yolk glands are present on both sides of the alimentary canal. The testis area is shorter in relation to the rest of the body than in *F. hepatica*.

The eggs are larger than those of *F. hepatica*, varying from 0.125 mm. to 0.175 mm. in length to 0.060 mm. to 0.100 mm. in breadth.

Habitat. Liver of ruminants.

Looss' observation that in this species we have to do with an African liver fluke in the same way as *F. magna* may be called an American fluke, must be looked upon with caution, until it is known whether the Rangoon specimens indicate a wider distribution than he had reason for suspecting.

FASCIOLA NYANZI (LEIPER) (Pl. III, figs. 3 and 4).

This fine species was found by Leiper in the bile ducts of the liver of a hippopotamus taken in Uganda and described by him under the above name (1910). His specimens were somewhat macerated and he was therefore unable to describe the species in every particular. Among the material from Nairobi (tube No. 65) I fortunately found a number of well preserved specimens of this species and I am able to add to Leiper's original description.

None of the specimens were as large as the largest described by Leiper, the longest being 59 mm. by 9 mm., the broadest 13.5 mm. by 49 mm. and the smallest specimen 35 mm. by 4.5 mm., but the remarkable general form was unmistakably as he described it. The species is especially noticeable for the great breadth of the "shoulders," the large cone and the long tapering body, almost entirely occupied by yolk glands.

The worm is thickly covered with spines, of a moderate size on the body and smaller and more closely set on the cone. The oesophagus is exceedingly short and the paired diverticula of the gut approximate behind the level of the shell gland and for the rest of their course lie very close together. The internal branches of the diverticula are fairly numerous, but small and infrequently branched. Branches from opposite sides frequently pass across one another or lie close together, giving a superficial appearance of anastomosis. The testes are confined to the anterior third only of the body. The ovary lies on the right hand side of the animal and is a typical branched body. The uterus is short and its coils are close behind the ventral sucker. Laurer's canal is present; it is very small and in the specimens examined had no contents. The yolk glands extend rather further forward into the "shoulders" in my specimens than in Leiper's, and lie exclusively (except for a stray branch or two) on the ventral side of the gut. It shares this interesting feature with *F. magna* in the genus *Fasciola*. The point is particularly easy to make out in the cleared specimens; viewed from the dorsal surface the alimentary canal is sharply outlined against a background of yolk glands.

The excretory and nervous systems are as in *F. hepatica*.

The eggs vary considerably in size in different specimens, and the size

given by Leiper (0.150 mm. by 0.070 mm.) must be taken as a minimum. Extracted from the uterus and measured in 70 per cent. alcohol the eggs lie between 0.150 mm. to 0.190 mm. in length and 0.070 mm. to 0.110 mm. in breadth. Measured from balsam preparations the eggs are on the average smaller (through shrinkage) than those extracted and measured in the medium in which the flukes are preserved.

SUMMARY.

1. The specimens in the material here dealt with make a series of forms connecting *F. angusta* (Railliet) and *F. aegyptiaca* (Looss).

2. No important character separates any of these flukes from *F. gigantea* (Cobbold) and they must accordingly be all identified with this species.

3. The following key represents the simplest method of distinguishing between the species of *Fasciola* at present described. (For detailed diagnoses see the literature referred to below and this paper.)

- | | | | |
|-----|---|--|---------------------|
| (1) | { | Yolk glands ventral to gut | 2. |
| | { | Yolk glands on both sides of gut. | 3. |
| (2) | { | Body very large and thick; cone not very distinct; oesophagus one and a half to three times as long as pharynx | <i>F. magna.</i> |
| | { | Body slender and awl shaped; cone very large and distinct; oesophagus exceedingly short | <i>F. nyanzi.</i> |
| (3) | { | Body at least three times as long as broad; "shoulders" absent or indistinct; internal branches of gut numerous and branched | <i>F. gigantea.</i> |
| | { | Body broad and leaf shaped; cone sharply set off from body on wide "shoulders"; internal branches of gut few and little if at all branched | <i>F. hepatica.</i> |

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EXPLANATION OF PLATE III.

The lateral extent of the yolk glands is shewn in each figure by shading. Only the broad plan of the external branches of the diverticula of the gut is indicated, the fine terminal arborisations being omitted.

- Fig. 1. *Fasciola gigantica* (Cobbold). Ventral view.
- Fig. 2. „ „ „ „ Ventral view.
- Fig. 3. *Fasciola nyanzi* (Leiper). Anatomy, from ventral view.
- Fig. 4. „ „ „ „ External features; ventral.

EXPLANATION OF LETTERING.

a.s.—anterior sucker and mouth; *ov.*—ovary; *ph.*—pharynx; *sh. gl.*—shell gland; *T.*—testis area; *ut.*—uterus; *v.s.*—ventral sucker; *y.g.*—yolk gland area.

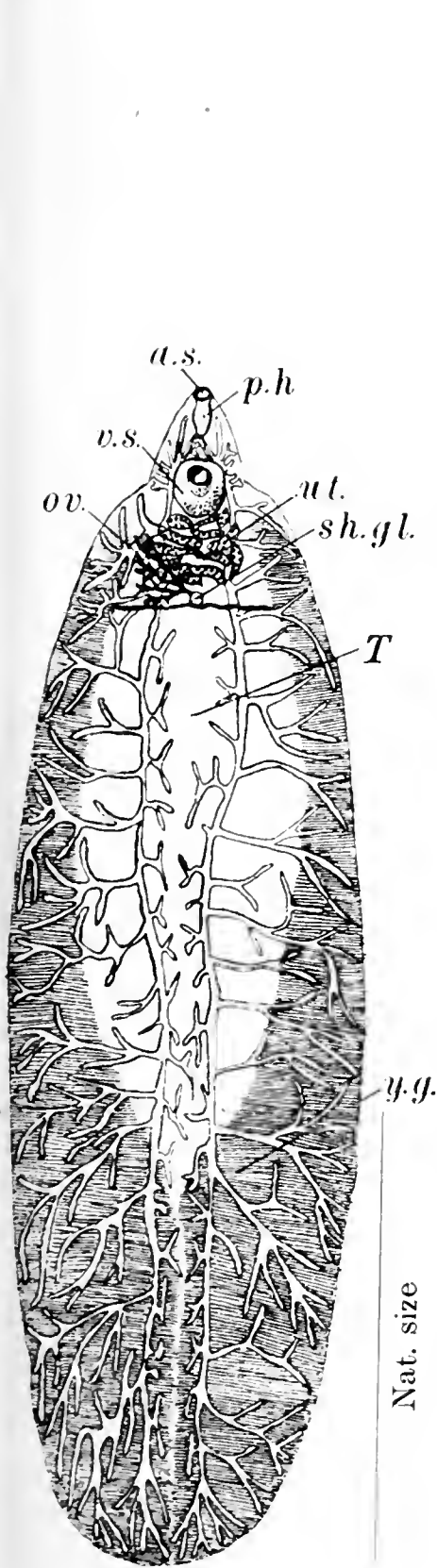


Fig. 1

Nat. size

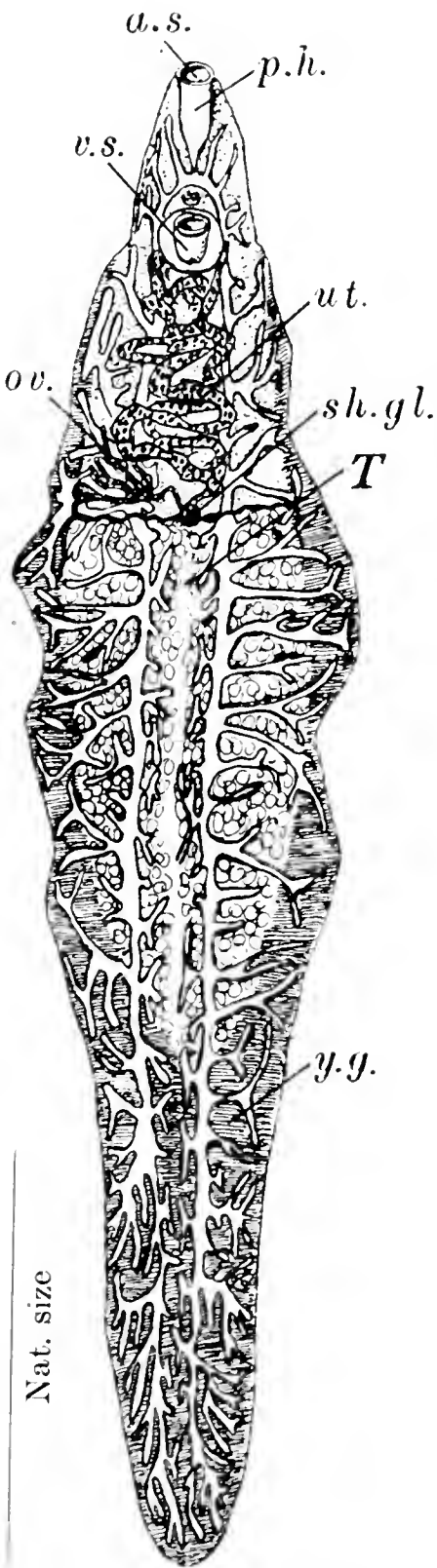


Fig. 2

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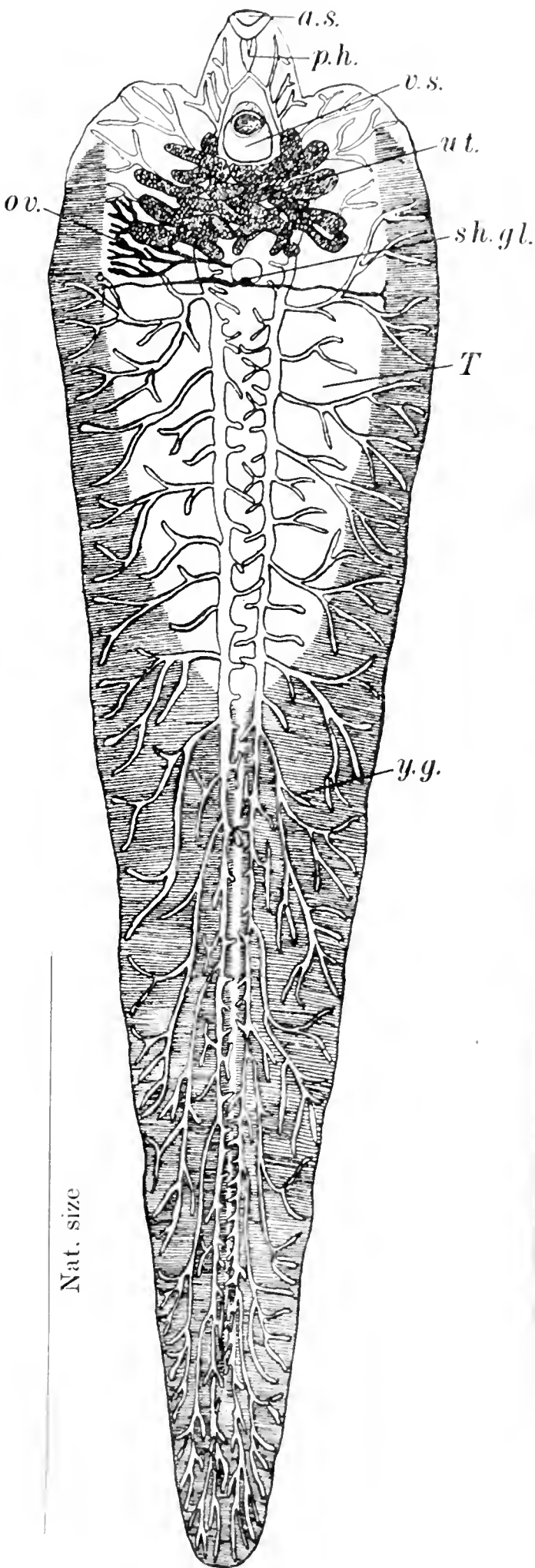


Fig. 3

Nat. size



Fig. 4

Nat. size

A NEW GENUS OF NEMATODES PARASITIC IN ELEPHANTS.

BY H. A. BAYLIS, M.A.

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(With 7 Text-figures.)

A RE-EXAMINATION of the type-specimens of "*Sclerostoma*" *clathratum* Baird from the African elephant, which are in the British Museum, has led to a rather interesting discovery. The material was contained in two bottles, labelled in Baird's own handwriting. One bore the name "*Sclerostoma clathratum* Baird," and contained a single male specimen of the form now known as *Grammocephalus clathratus*. The other bottle was labelled "*Sclerostoma clathratum* Baird, ♀," and proved to contain worms of both sexes and of quite a different type from *Grammocephalus*.

It is somewhat remarkable that the curious figure given by Baird (1868) of the head of *Sclerostoma clathratum* has called forth little or no comment from subsequent writers. The fact appears to be that Baird had confused two quite distinct species, belonging to widely different genera, and the figure of the head (*l.c.* Fig. 2 *a*) as also the figures of the supposed female, do not belong to the same form as the enlarged figure (2 *c*) of the male tail, which is that of the *Grammocephalus* of recent authors.

The species taken for the female of *S. clathratum* by Baird appears to be a worm of Spirurid affinities, and is clearly very closely related to the "*Filaria*" *smithii* of Cobbold (1882), from the Indian elephant. Unfortunately it has not been possible to procure specimens of the latter form for comparison; but through the kindness of Lt.-Col. Clayton Lane material belonging to a very similar species from the Indian elephant has been placed at the writer's disposal, and on comparison of this and Baird's material with Cobbold's description and figures it seems clear that all three species belong to one and the same genus.

Railliet, Henry and Bauche (1914) have noted the Spirurid affinities of *Filaria smithii*, and have named it provisionally *Spiroptera smithii*, using the name *Spiroptera* in default of a clearer definition of its position. The genus to which these worms belong seems to the writer to have very close relationships with *Habronema*, Diesing, and may be defined as follows:

PARABRONEMA, n.g.

Spiruridae: Polymyarian worms, having the mouth bordered by paired lateral lips, externally to which there are a dorsal and a ventral shield of cuticle. Each lip bears one large, median lateral papilla and a pair of small sublateral papillae. Of the dorsal and ventral shields, each carries a pair of larger papillae (Figs. 1, 4, *p.*), situated at some distance behind the extremity of the head. The cuticle of the head is thick, and folded in a complicated manner so as to form a circlet of six horseshoe-shaped auricular appendages (Figs. 1, 4, *a.*), of which two are lateral, two subdorsal and two subventral. The open anterior end of the horseshoe is partly filled up by a vesicular swelling of the cuticle. The edge of each of these appendages is further folded to form a groove somewhat resembling the cervical grooves or "cordons" of the *Acuaria* group.

The body is rather slender, tapering rather more in front than behind. The cuticle shows more or less distinct transverse striations. The buccal cavity, at a short distance from the oral aperture, is elongated transversely in a direction at right angles to the sagittal plane, and then passes into a long, cylindrical, cuticular tube (Figs. 1, 4), with thick walls. The oesophagus (Fig. 1, A) consists of a short, narrow, anterior portion and a long, somewhat wider, posterior portion, both portions being muscular. The anterior portion is surrounded by the nerve-ring. The minute excretory pore is situated at about the level of the nerve-ring, and a pair of cervical papillae (Fig. 1, A, *c.p.*) with bristle-like terminations a little further back.

The tail of the male is coiled ventrally into a spiral, provided near the extremity with lateral alar expansions, and largely covered on the ventral surface with interrupted, longitudinal cuticular ridges (Fig. 2). The spicules (Figs. 3, 6) are markedly unequal, the slender left spicule being from two to four times as long as the right, which is rather stouter. A small, somewhat triangular accessory piece is present (Figs. 3, 6). The paired caudal papillae have long nervous pulps, and are present to the number of six pairs, of which four are preanal and two postanal. The arrangement of the corresponding papillae of the two sides is somewhat asymmetrical. The anterior pair of postanal papillae (Figs. 2, 5) present a remarkable peculiarity, lying transversely across the ventral surface, the left a little in front of the right, with their terminations either passing beyond, or at least close to, the mid-ventral line of the tail. In addition to the paired papillae, a large median double papilla is present immediately in front of the cloacal aperture.

The female is considerably larger than the male. The tail is short, conically pointed or blunt, and characteristically curved towards the dorsal side. The vulva is situated almost immediately behind or in front of the posterior end of the oesophagus. The vagina is long, narrow and muscular, and runs straight backwards with the exception of a curious little U-shaped bend in its course (Fig. 7) at a short distance from the vulva. The two uterine branches are

parallel, running backwards at first, one of them returning towards the anterior end of the body, the other continuing posteriorly. The worms are apparently viviparous, the uterus containing immense numbers of embryos which are not enclosed in a hard egg-shell.

Hab. stomach[-wall] of elephants.

1. ***Parabronema indicum***, sp.n. (Genotype.)

(Figs. 1-3.)

For measurements see Table, p. 65.

This is a rather small form, in which the head (Fig. 1) is of a conical shape, distinctly narrower in front than at the back of the auricular appendages. The latter (Fig. 1, *a.*) are regularly horseshoe-shaped, and bear a narrow groove on their free edges. The borders of the lips (Fig. 1, *C*) are simple, without a well-marked median inward projection. The anterior pair of postanal papillae (Fig. 2) in the male overlap in such a way that the termination of the right papilla is well to the left of the mid-ventral line. The posterior pair of postanal papillae are symmetrically placed opposite to each other. The two terminations of the large median preanal papilla appear to be situated near its lateral limits. The left spicule is very slender, and is nearly three times as long as the right.

The female is about twice as large, on an average, as the male. The tail is conical, with rather blunt tip. A pair of caudal papillae are just visible as minute dimples in the cuticle close to the tip. The vulva is situated a little behind the posterior end of the oesophagus. The vagina forms a U-shaped bend at about 0.25 mm. from the vulva. After a further course of about 0.4 mm. it widens into a fusiform swelling, which gives off the two uterine branches posteriorly. The uterine branches themselves also have small fusiform enlargements near their origin. One branch runs back to a point about 0.35 mm. from the anus, where it turns forward again. The other turns forward sooner, and runs up nearly as far as the posterior end of the oesophagus, where it forms a loop and runs once more posteriorly.

This species differs from *P. smithii*, as described by Cobbold and by Mitter (1912), chiefly in its somewhat larger size, in the greater absolute length of the spicules of the male, and in the greater relative length of the left spicule. It also differs in the number and arrangement of the caudal papillae of the male; but it may be taken as almost certain that some of these papillae were overlooked by Cobbold, and that a re-examination of the species would show that their arrangement agrees with that in the forms here described.

Host. The type-material, now in the British Museum (Natural History), was collected from the stomach-wall of an Indian elephant (*Elephas indicus*)¹ by the Superintendent, Civil Veterinary Department, Madras.

¹ The writer is informed by Mr Oldfield Thomas, F.R.S., that the more correct name of the Indian elephant is *E. maximus*, and that of the African elephant *Loxodonta africana*. In the present paper, however, the more familiar names have been retained.

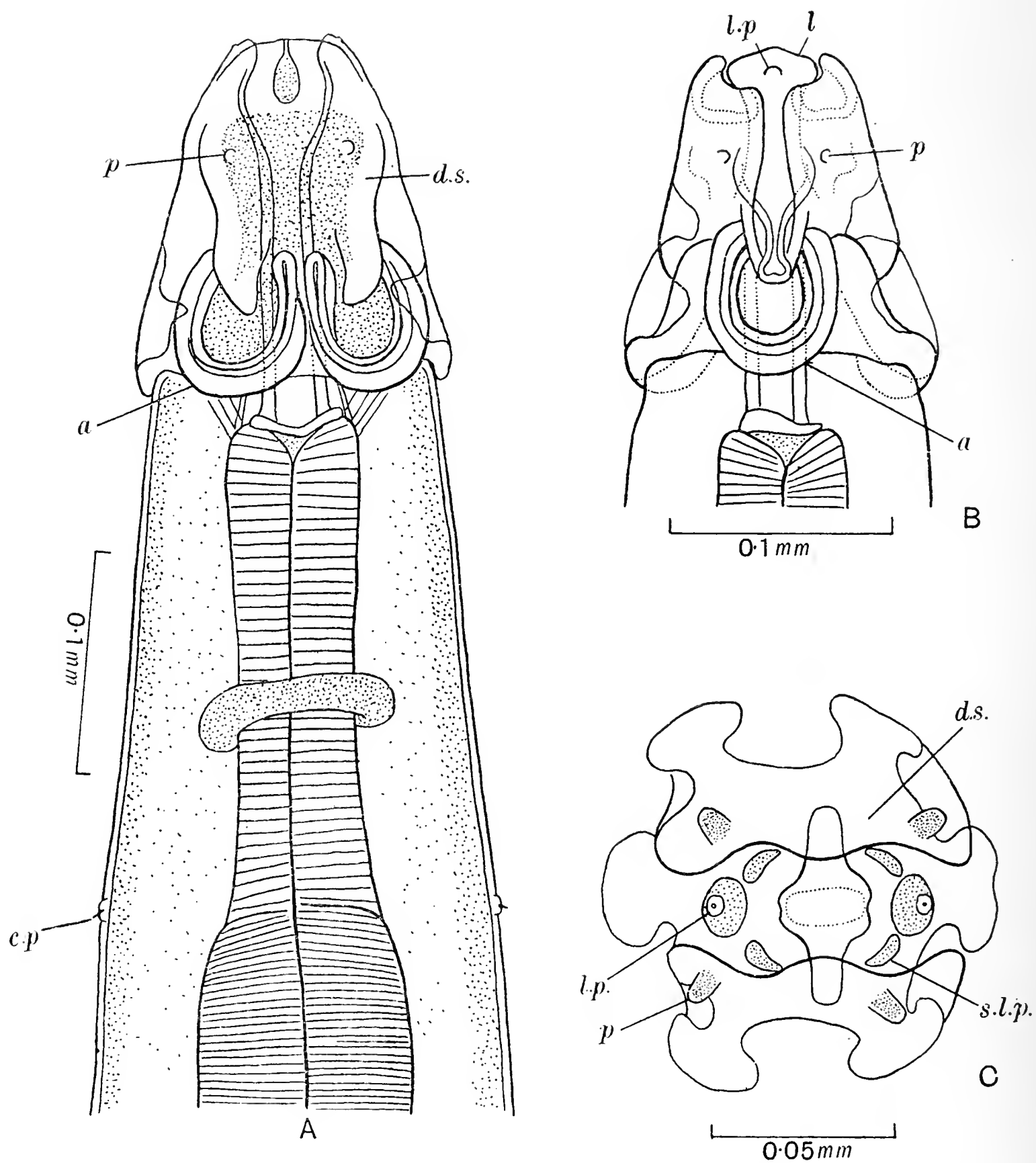


Fig. 1. *Parabronema indicum*. A, dorsal view of head and neck; B, lateral view of head; C, extremity of head, seen from the front. *a.*, *a.*, auricular appendages; *c.p.*, cervical papilla; *d.s.*, dorsal shield; *l.*, lip; *l.p.*, lateral papilla of lip; *p.*, *p.*, papillae of dorsal and ventral shields; *s.l.p.*, sublateral papilla of lip.

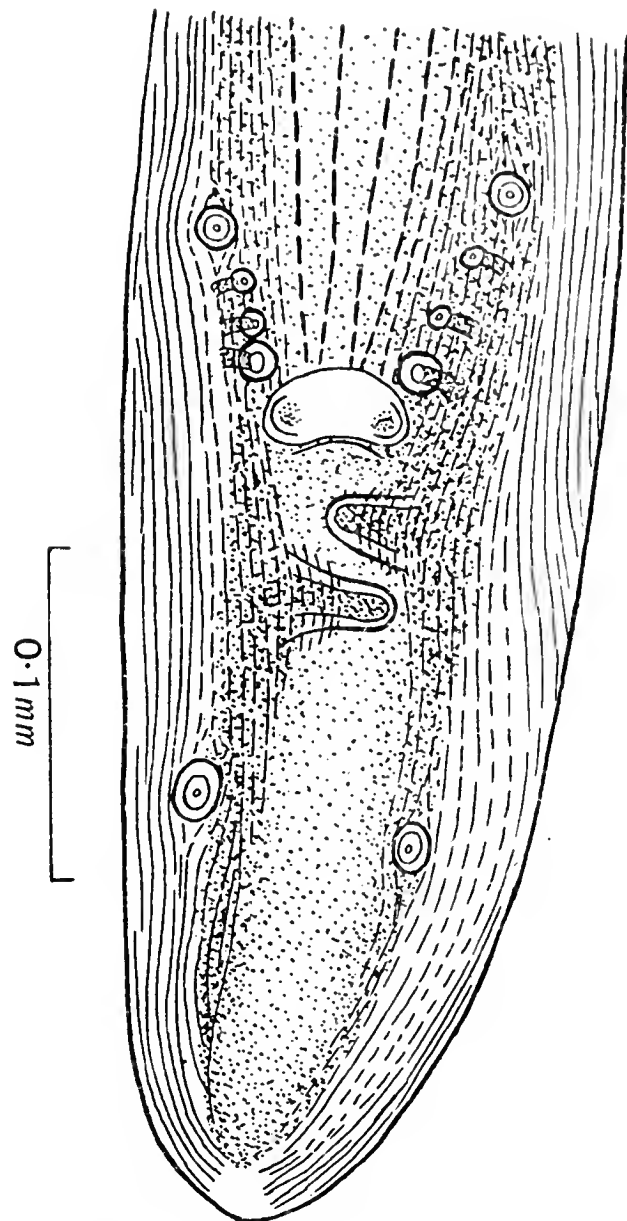


Fig. 2. *Parabronema indicum*. Tail of male; ventral view.

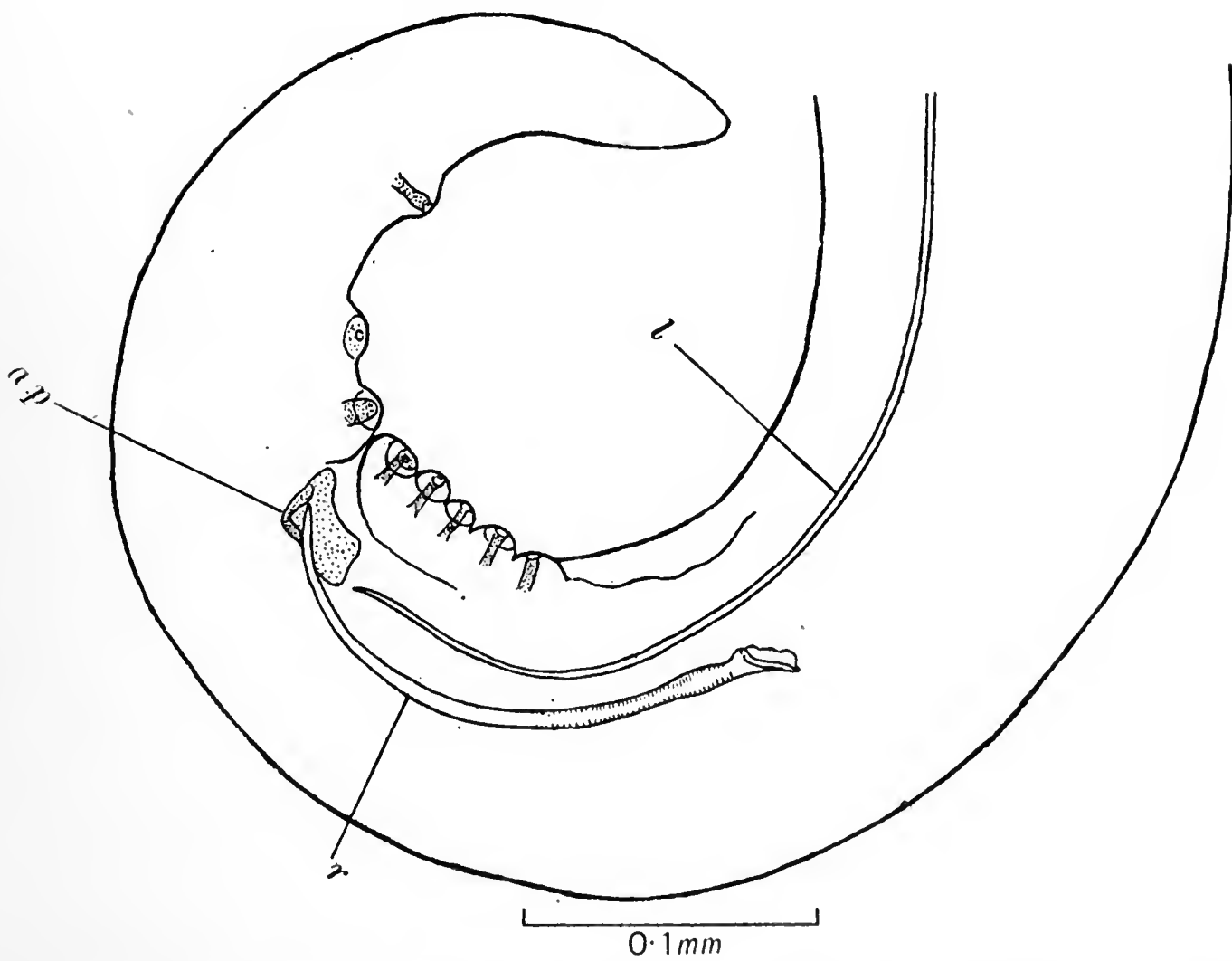


Fig. 3. *Parabronema indicum*. Tail of male; lateral view. *a.p.*, accessory piece; *l.*, left spicule (terminal portion); *r.*, right spicule.

2. *Parabronema africanum*, sp.n.

(Figs. 4-7.)

Sclerostoma clathratum (♀) Baird (1868), p. 262; Figs. 2 ♀, 2 a, 2 b.

For measurements see Table, p. 65.

This is a much larger form than the preceding, and the inequality in size between the sexes is less marked, the male attaining about two-thirds of the

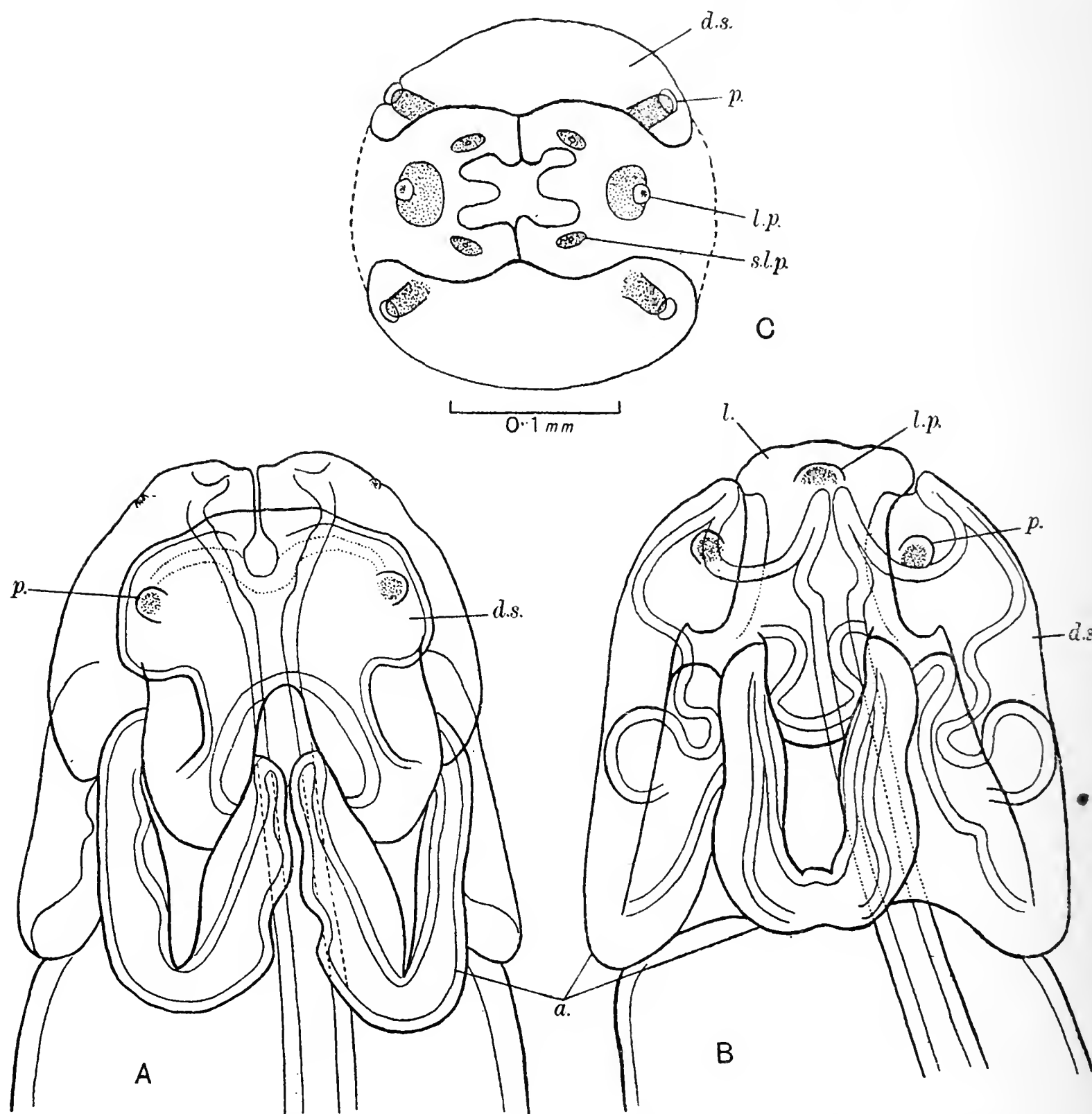


Fig. 4. *Parabronema africanum*. The head. A, dorsal view; B, lateral view; C, the extremity, seen from the front. a., auricular appendages; d.s., dorsal shield; l., lip; l.p., lateral papilla of lip; p.p., papillae of dorsal and ventral shields; s.l.p., sublateral papilla of lip.

length of the female. The male, however, is easily distinguished by the well-marked spiral coiling of the tail, and it is rather astonishing that Baird did not suspect an error when he described the tail of the female *S. clathratum* as "obtusa, saepe convoluta."

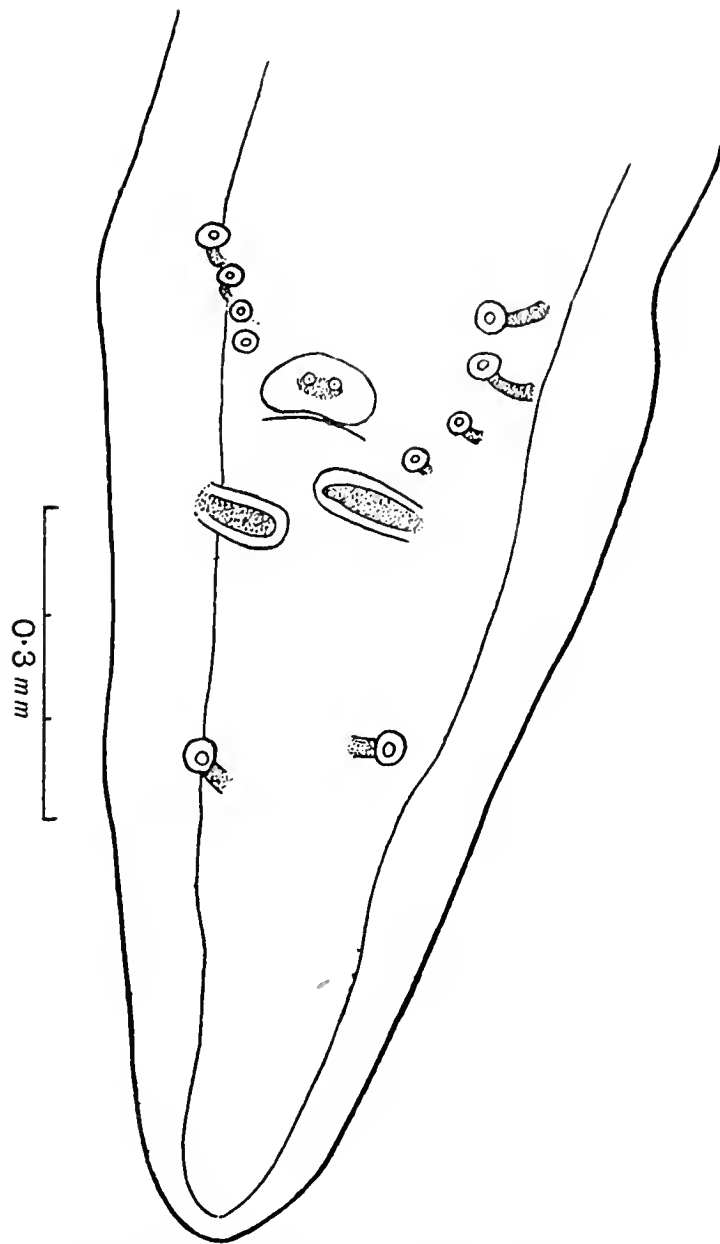


Fig. 5. *Parabronema africanum*. Tail of male; ventral view.

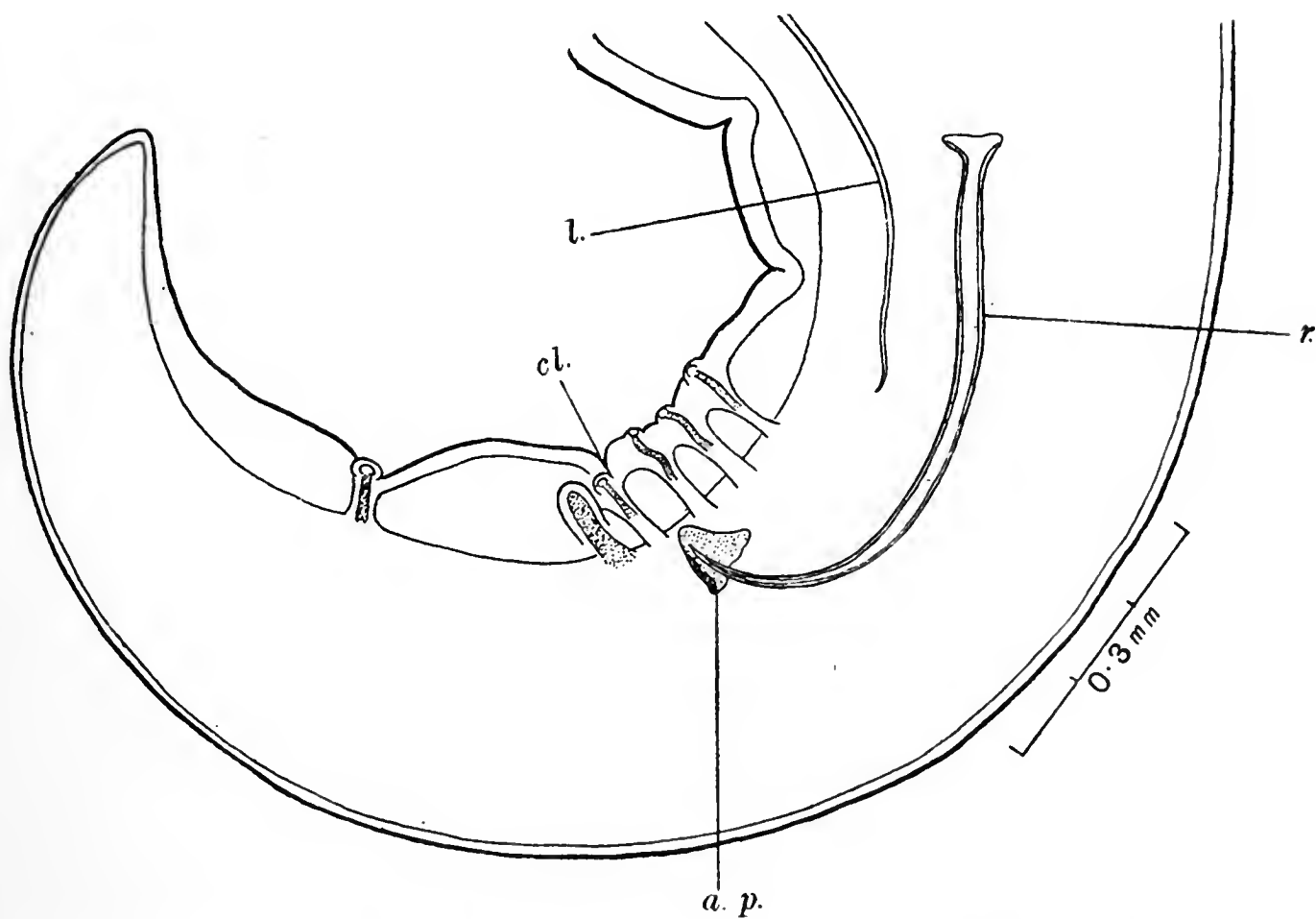


Fig. 6. *Parabronema africanum*. Tail of male; lateral view. *a.p.*, accessory piece; *l.*, left spicule (terminal portion); *r.*, right spicule.

The cuticle, especially near the head, shows the minute longitudinal striations, in addition to the usual transverse striations, which suggested to Baird the name *clathratum*.

The head (Fig. 4) is almost invariably bent towards the dorsal side, and may be roughly described as bullet-shaped. It is nearly as wide at the level of the outer papillae as at the back of the auricular appendages. The latter (of which there are six, and not five, as stated by Baird) are somewhat elongate, with their edges curled inwards so as to form a nearly V-shaped aperture, and their "grooves" are very wide, and carried on the inner surface.

The lips (Fig. 4, C) have a median inward projection on their opposed edges, with deep incisions on either side of it, so that the outline of the mouth is somewhat X-shaped. The cervical papillae are situated in little cuticular pits, from which their minute bristle-like terminations project.

The caudal papillae of the male (Figs. 5, 6) are distinctly asymmetrical, the group of four preanal papillae on the left side being much more widely separated than those on the right, so that the most posterior of the group lies at the level of the cloaca. The terminations of the anterior postanal

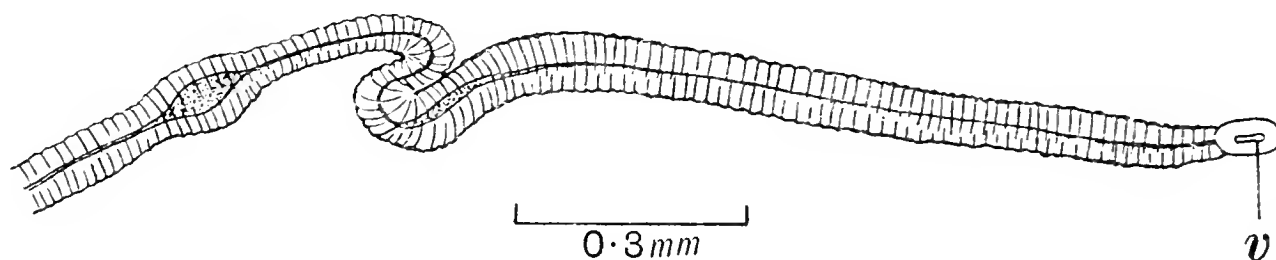


Fig. 7. *Parabronema africanum*. Terminal portion of vagina. *v.*, vulva.

papillae do not quite reach the mid-ventral line of the tail. The two terminations of the median preanal papilla appear to be situated close together near the middle line. The left spicule is more than four times as long as the right.

The tail of the female is conical, with a rounded tip. Caudal papillae have not been observed. The vulva is situated either just behind or just in front of the posterior end of the oesophagus. The U-shaped bend of the vagina (Fig. 7) occurs at about 1–1.2 mm. from the vulva. Behind this point the vagina becomes narrower, still running straight backwards, and gradually widening, for a further distance of about 3.2 mm. before giving off the uterine branches, the course of which is probably similar to that described for *P. indicum*.

Host. The type-material was collected by Dr Murie from the stomach of a young African elephant (*Elephas africanus*) which died in London in 1867.

3. *Parabronema smithii* (Cobbold).

Filaria smithii Cobbold (1882), p. 237; Text-figs. 6–7; Pl. XXIV, figs. 7–10.

Filaria smithii Mitter (1912), p. 113; Pl., Figs. *e*—*g*.

Spiroptera smithi Railliet, Henry and Bauche (1914), p. 210 (*nec Filaria smithi* Sambon, 1907).

For measurements see Table, p. 65.

Practically the only distinctive features for this species that can be gathered from Cobbold's description are those of size. As has been pointed out above,

when dealing with *P. indicum*, the number and arrangement of the male caudal papillae cannot, probably, be regarded as satisfactorily described. The two rows of translucent auriculate appendages of the head referred to by Cobbold may probably be interpreted as corresponding to (a) the auricular appendages themselves, and (b) the vesicular swellings of the cuticle which partly fill their cavities in front, in the two forms now described. The account given of the cephalic papillae is not very clear, while nothing corresponding to the minute papillae said to exist within the concavity of the auricular appendages has been observed in *P. indicum* or *P. africanum*. Mitter (1912) adds little to Cobbold's description.

Host. Cobbold's material was collected from the coats of the stomach of *Elephas indicus*, where the worms inhabited tumours of about the size of a filbert nut and of an oval, flattened shape. A similar habitat is indicated by Mitter, who, however, mentions the "intestines," not the stomach.

Table of measurements of the species of Parabronema.

(All measurements are in millimetres, and represent specimens of about the maximum size. The figures for *P. smithii* have been calculated from the fractions of an inch given by Cobbold, and are only approximate.)

	Species					
	<i>indicum</i>		<i>africanum</i>		<i>smithii</i>	
	♂	♀	♂	♀	♂	♀
Length	7.9	13.0	40.0	57.0	4.2*	8.5*
Thickness (maximum) ...	0.31	0.39	0.8	1.0	0.2	0.25
Diameter of head at posterior end of auricular appendages	0.14	0.15	0.27	0.32	—	—
Length of tail... ..	0.17	0.32	0.85	0.52	—	—
Length of buccal cavity ...	0.17	0.18	0.35	0.4	—	—
Distance from anterior extremity to posterior end of auricular appendages ...	0.15	0.17	0.3	0.36	—	—
Distance from anterior extremity to posterior end of cervical papillae	0.39	0.42	0.7	0.85	—	—
Distance from nerve-ring ...	0.32	0.34	0.7	0.83	—	—
Distance from excretory pore	0.33	0.35	?	0.9	—	—
Distance from junction of two divisions of oesophagus ...	0.4	0.4	0.9	1.0	—	—
Distance from posterior end of oesophagus	2.2	2.25	6.5	7.2	—	1.5
Distance between transverse striations of cuticle ...	0.0035		0.01		—	
Length of spicules	{ R. 0.39 L. 0.93		R. 0.68 L. 3.15		Shorter	0.18
Greatest length of accessory piece... ..	0.06		0.08		Longer	0.32
Distance of vulva from posterior end of oesophagus	0.4		0.1 in front, 0.2 behind [Probably about 0.2 behind]			
Distance from caudal papillae (♀) to tip of tail	0.03		—		—	

* Mitter (1912) gives 4.16 mm. for the length of the male and 8.3 mm. for that of the female of *Filaria smithii*.

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SOME OBSERVATIONS AND EXPERIMENTS ON INSECT FLAGELLATES, WITH SPECIAL REFERENCE TO ARTIFICIAL INFECTION OF VERTEBRATES.

(A REPORT TO THE DEPARTMENT OF SCIENTIFIC AND
INDUSTRIAL RESEARCH¹.)

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(From the Wellcome Bureau of Scientific Research.)

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I. OBSERVATIONS ON *CRITHIDIA MELOPHAGIA* AND ITS RELATION TO THE SHEEP-TRYPANOSOME.

WOODCOCK (1910) discovered a trypanosome naturally occurring in the blood of British sheep. This finding was confirmed by Behn (1911, 1912) in Germany. In both cases the authors did not give any description of the parasite, which was seen by them only in single cases, and they failed to cultivate the trypanosomes.

According to Woodcock (1910) the trypanosome represents a developmental stage of *Crithidia melophagia* Flu, 1908, parasitising the gut of the sheep-ked, *Melophagus ovinus*. As the "ked" is a typical blood-sucking insect, and the degree to which it may be infected with these flagellates

¹ The observations here described were made at the Wellcome Bureau of Scientific Research with the aid of a grant from the Department of Scientific and Industrial Research. I take this opportunity of offering my warmest thanks to the Director of the Bureau, Dr Andrew Balfour, C.B., C.M.G., for his hospitality and kind assistance, and to Dr C. M. Wenyon, C.M.G., C.B.E., M.B., B.Sc., who has given me unfailing help and valuable advice in the course of my work. I am also indebted to Dr Wenyon for performing the inoculations for me, as I had not at that time obtained a Licence for such experiments.

frequently reaches 100 per cent., such a supposition was quite natural, although direct evidence of any connection between the said flagellate and the sheep-trypanosome was lacking.

Later, the morphology and life-history of *Crithidia melophagia* in the gut of the sheep-ked were described in detail by Swingle (1909) and Porter (1910), who arrived at the conclusion that it was a specific insect parasite. This seemed the more obvious to the authors since both they and Flu (1908) had discovered that infection in the "keds" was transmitted hereditarily, so that the necessity of a second host, the sheep, seemed to have been eliminated.

Swingle (1911, 1911*a*) tested this view experimentally. He examined the blood of a great number of sheep; inoculated them with, and fed them on, the contents of sheep-keds' guts infected with these flagellates, and put freshly hatched "keds" on sheep previously washed in an antiseptic solution. All these experiments gave negative results, with the exception of two cases, in which young "keds" which showed no flagellates became infected with them after having lived on sheep for twelve days. This occurrence, however, the author explained as hereditary infection of the "keds."

Swingle's experiments were conducted so exhaustively that the question regarding the presence of trypanosomes in sheep and their relation to the flagellates of the sheep-ked seemed to have been finally solved.

There remained, however, several points which were not quite clear, and there were other methods by which the problem could be further investigated.

The first point that required further elucidation was the alleged hereditary transmission of *C. melophagia*. Hereditary infection was described by Flu (1908), Swingle (1909) and Porter (1910) as the means by which infection is maintained in the sheep-keds.

The second question to be solved was the possibility of cultivating the supposed trypanosomes from the sheep's blood. The negative results of numerous blood examinations made by Pfeiffer (1905), who was the first to describe *C. melophagia* Flu (1908), Swingle (1911, 1911*a*), and Porter (1910), and the scanty positive results obtained in the same way by Woodcock (1910) and Behn (1911, 1912) showed that, even if trypanosomes occur in the sheep's blood, their number is so small that in most cases they escape detection. Now, it was shown by Crawley (1909, 1909*a*, 1912) that it is possible to cultivate trypanosomes from the blood of cattle in which the ordinary examination of blood failed to reveal their presence. Stockman (1910) also discovered trypanosomes in British cattle which had hitherto escaped detection, and the observation has been confirmed by other authors.

Having these points in view, and taking into account the general importance of this question, both from the theoretical and practical aspect, since the presence of even a slight trypanosome infection might, under favourable conditions, be capable of producing a serious disease in sheep, as in the case of other domestic animals similarly infected, I have endeavoured to test this question experimentally.

Unfortunately, on account of the difficulty of obtaining live "keds," owing to the operation of the dipping laws in England, my experiments could not be conducted on as wide a scale as desired, but had to be limited to the study of certain points in the life-history and behaviour of *C. melophagia* and to attempts at culture of trypanosomes from the sheep's blood, and artificial infection of other mammals with these flagellates.

Whilst this work was in progress, several publications by Nöller (1917, 1919, 1919 *a*, 1920) came to hand from Germany. The author appears to have been studying this subject for several years. By using special culture media and methods, he succeeded not only in cultivating trypanosomes from the sheep's blood, but also in bringing about the transition from the crithidia forms to trypanosomes *in vitro*, thus establishing the identity of the two forms without any doubt. The trypanosome received the name of *Trypanosoma melophagium* Flu, 1908.

I had at my disposal four sheep¹, from which blood-cultures were made and kept at 24° C. and at 30° C., according to Nöller's methods (1919, 1919 *a*) in slightly alkaline bouillon. I did not succeed in cultivating any trypanosomes from the blood of these sheep, although they were infected with "keds" containing flagellates in their guts. As it was impossible to obtain new material, and especially very young lambs, experiments on which might have been more fruitful, I was compelled to postpone this part of the work in the hope of being able to continue it early in the spring, when conditions are more favourable.

As the morphology and development of *C. melophagia* in the gut of *Melophagus ovinus* have been studied and described in detail by other authors, I have directed my attention only to certain points that remained obscure in their description. One question is of especial importance. Flu (1908), Swingle (1909, 1911) and Porter (1910) claimed that these flagellates are transmitted to their offspring hereditarily, the flagellates penetrating into the ova, and thence into the developing embryo. The weak point in their description is the fact that the forms described and depicted by the three authors named have nothing in common with each other. Each author gives a different picture of the "hereditary" forms found, and with the exception of Porter's figures, most of them bear no resemblance to any of the forms which insect- or haemoflagellates usually assume. Porter claims to have actually seen the flagellate forms of the Crithidia penetrating through the eggs of the "ked," losing their flagella there and passing into the "resting stage."

I have examined a fair number of ova and pupae of the sheep-ked both in sections and in smears, and have employed Porter's technique in the latter case, but have failed to find any forms which could be interpreted as stages of a flagellate organism. In this respect my observations agree with those of Chatton and Delanoë (1912) and Cauchemez (1912), who have also failed to

¹ I wish to acknowledge my indebtedness to Mr J. B. Buxton, F.R.C.V.S., of the Wellcome Physiological Research Laboratories, for kindly supplying me with the necessary material for my work.

find any traces of hereditary infection in the sheep-ked. In several cases I saw structures which at first glance could be mistaken for flagella of the parasite, which, according to Porter, "are found floating freely in the vitellus of eggs." A careful examination of these structures, however, convinced me that they were spermatozoa, which find their way into the ovaries from the *receptaculum seminis*. It is a noteworthy fact that, although the authors named believed that hereditary transmission exists, they have all failed to find any traces of the flagellates thus inherited either in the larvae or in newly hatched "keds." Only after prolonged feeding on sheep's blood do the flagellates make their appearance in the gut of the young "keds." The natural deduction from such a fact would be the assumption of a possibility, at least, of infection from the sheep's blood, but the authors' conviction of the occurrence of "hereditary infection" leads them to suppose that in the larvae and newly hatched "keds" the flagellates are present in a cryptic stage, which is stimulated to final development by the ingestion of sheep's blood.

In order to throw some light on the relationship between *C. melophagia* and the sheep, I have made attempts artificially to infect mice with them.

In the course of these experiments I became interested in this question in general, and have extended the experiments to other vertebrates, using flagellates parasitising other insects also. These experiments are recorded in the second part of this paper, together with some observations on the vitality of *C. melophagia in vitro*.

II. EXPERIMENTS ON ARTIFICIAL INFECTION OF DIFFERENT VERTEBRATES WITH INSECT FLAGELLATES.

The possible pathogenicity of insect flagellates of the *Herpetomonas* type for vertebrates in which they do not naturally occur is interesting from several points of view. It may, on the one hand, throw light on the question of the phylogeny of the pathogenic haemo-flagellates—the trypanosomes and leishmanias; on the other hand it may serve to elucidate to a certain degree the rôle of insects as carriers and intermediate hosts in certain diseases in which such have not hitherto been found, *e.g.* in Kala-azar and Oriental Sore.

That trypanosomes and leishmanias have originated from purely insect flagellates which, by association of their hosts with vertebrates have gradually adapted themselves to life in the latter, is a hypothesis which, as far as trypanosomes are concerned, finds confirmation in the fact that the latter usually pass part of their life-cycle in some insect, the insect stages being similar to the forms of purely insect flagellates of the *Herpetomonas* group. As regards leishmanias, there is no direct evidence as yet of their connection with such forms, the only evidence of their relationship to the Herpetomonads being the flagellate forms they produce in cultures, the fact that they develop into flagellates in the stomachs of certain insects (Wenyon, 1911), and the very rare occurrence of such forms in the body of their vertebrate host (cf. Wenyon, 1914, 1915).

Recently, numerous works have appeared dealing with artificial infection of vertebrates with the flagellates of different insects both associated and unassociated with vertebrates. The majority of these are by Laveran and his collaborators, and Fantham and Porter. These observers claim to have proved that insect flagellates, when introduced into vertebrates, evoke in the latter symptoms of disease comparable to Leishmaniasis, and assume in them forms similar to the leishmania parasites.

I have repeated some of the experiments mentioned, and conducted others with new flagellates, but have failed entirely in producing any form of disease, or in finding any forms of the flagellates introduced into the animals experimented upon. These experiments have, however, led to certain observations on the vitality of the parasites thus introduced.

As the question of such artificial infection is of considerable interest, and important theoretical and practical conclusions have been deduced from them by the authors mentioned above, I propose to give a short description of my experiments, together with a brief review of the results arrived at by these authors.

The following are the species of flagellates with which such experiments had been made:

- | | |
|--|------------------------------------|
| 1. <i>Herpetomonas pattoni</i> . | 8. <i>H. stratiomyiae</i> . |
| 2. <i>H. ctenopsyllae</i> . | 9. <i>H. pediculi</i> . |
| 3. <i>H. ctenocephali</i> . | 10. <i>Crithidia fasciculata</i> . |
| 4. <i>H. phlebotomi</i> . | 11. * <i>C. melophagia</i> . |
| 5. <i>H. muscae-domesticae</i> . | 12. <i>C. tabani</i> (?) |
| 6. ** <i>H. calliphorae</i> ¹ . | 13. <i>C. gerridis</i> . |
| 7. * <i>H. jaculum</i> . | |

For the sake of convenience I have reproduced, in Table I, the methods and results of experiments on artificial infection of vertebrates by various authors. As far as I could ascertain, this Table contains all the chief experiments on this subject (with the exception of some similar experiments on birds).

It can be seen from this Table that some of the workers have succeeded in introducing flagellates from insects into various vertebrates, and have evoked in them infections similar to Leishmaniasis, both as regards the morphology of their parasites and, in some cases, the lesions as well. Successful results were obtained with flagellates both from insects which are naturally associated with vertebrates and those which have no such association.

In the animals thus infected, the flagellates are said to make their appearance in the blood and organs in the form of leishmania and flagellate bodies. Infection of mice frequently terminates in death, and examination of organs *post mortem* shows that some of them (spleen and liver) are hypertrophied.

¹ Asterisks denote species which were used in my experiments as well; the species marked with two asterisks had not been used by previous workers.

Table I.

Experiments of different authors on artificial infection of vertebrates.

Species of flagellate	Host	Experimental animal	Mode of infection	Author	Results	Forms of parasites described in infected animals	Condition of infected animal	Remarks
1. <i>H. pattoni</i>	Rat-flea	Mice and Rats	per os	Laveran and Franchini, 1914 1914 <i>a</i>	+	Intracellular flagellates, free flagellates and leishmania forms in blood, liver, spleen and bone-marrow	—	Amongst the parasites were found leishmania forms with one nucleus and fusiform aflagellate forms. Cultures successful
2. <i>H. ctenu-psyllae</i>	"	Mice	"	<i>Ibid.</i> 1914 <i>b</i>	+	Leishmania forms in blood and organs	—	Uninucleate leishmania forms found in blood and organs
3. <i>H. ctenu-cephali</i>	Dog-flea	(a) Guinea-pigs and mice	i.p. (cultures)	<i>Ibid.</i> 1920	+	Leishmania forms in blood and organs	Mouse infected from guinea-pig, died	Successful passage from guinea-pig to mouse
		(b) Mice	i.p.	<i>Ibid.</i> 1913	+	Leishmania forms in blood and peritoneal exudate	—	Most of the leishmania forms extra-cellular. Passages successful. Duration of infection 60 days
		(c) Mice, rat	i.p., s.c., intraven.	<i>Ibid.</i> 1913 <i>a</i>	+	Leishmania forms in blood and organs	Mice very weak, some died from infection. Spleen hypertrophied	Passages (3) successful
		(d) Mice	i.p.	<i>Ibid.</i> 1919	75 % +	"	—	Flagellates produced in cultures
		(e) Mice (f) Mice	i.p. i.p. (culture)	Chatton, 1919 Laveran and Franchini, 1919 <i>a</i>	— 90 % +	— Leishmania forms free and intracellular, and flagellates	— Three died. Emaciation, diarrhoea. Liver and spleen hypertrophied	Cultures negative Virulence increased by passages
		(g) Mice (h) Dog	(Culture) per os	Nöller, 1920 Fantham and Porter, 1915	— +	— —	— —	— —
		(i) Dogs	By placing dogs together with fleas	Wenyon, 1914	—	—	—	Dogs died from the effects of flea-bites

Species of flagellate	Host	Experimental animal	Mode of infection	Author	Result	Forms of parasites described in infected animals	Condition of infected animal	Remarks
4. <i>H. phlebotomi</i>	Sandfly	Dogs and guinea-pigs	s.c., i.p.	Laveran and Franchini, 1920	+	Leishmania forms free and intracellular, and flagellates	Symptoms of Kala-azar and Oriental Sore	—
5. <i>C. fasciculata</i>	Mosquito (<i>A. maculipennis</i>)	Mice and rats	?	<i>Ibid.</i> 1914, 1913 <i>a</i>	+	Leishmania forms	Symptoms of Oriental Sore	Parasites very rare. Successful passages
6. <i>C. melophagia</i>	Sheep-ked	Mice	(<i>a</i>) i.p. (cult.) (<i>b</i>) per os	<i>Ibid.</i> 1919 <i>a</i> , 1914 <i>b</i>	+	(<i>a</i>) Leishmania forms free and intracellular, and flagellates in blood and organs	—	(<i>a</i>) Virulence increased by passages
7. <i>C. tabani?</i>	<i>Tabanus socius</i>	Rats	i.p.	Wenyon, 1908	—	—	—	—
8. <i>H. pediculi</i>	<i>P. vestimenti</i>	Mouse	per os	Fantham and Porter, 1915 <i>a</i>	+	—	—	—
9. <i>H. muscae-domesticae</i>	Housefly	Mice and rats	i.p.	Franchini and Mantovani, 1915	+	Free leishmania forms in blood. Leishmanias in organs	Rat died "brusquement" after 3 months	Leishmania forms frequently with one nucleus. Rat's blood in culture produced "anaplasma" bodies which produced rare leishmania bodies in the liver of a mouse, when inoculated with it
10. <i>H. jaculum</i>	<i>Nepa cinerea</i>	(<i>a</i>) Mice	per os, i.p.	Fantham and Porter, 1915	+	Rounded forms in blood after a few hours. Leishmania forms and flagellates in organs	Mice weak, died, or killed <i>in extremis</i>	Parasites few
		(<i>b</i>) Sticklebacks	s.c., per os.	<i>Ibid.</i> 1915 <i>a</i>	+	Flagellates in blood, leishmania and flagellate forms in organs	Swelling at the site of inoculation	—
		(<i>c</i>) Toad	?	"	+	—	—	—
		(<i>d</i>) Newt	per os	"	?	—	—	Newt accidentally lost
		(<i>e</i>) Water-snake	?	"	+	—	—	—
11. <i>C. gerridis</i>	Gerris	(<i>a</i>) Rana	i.p.	"	+	Leishmania forms in blood, same and Crithidia in liver	—	—
		(<i>b</i>) Lacerta	i.p.	"	+	—	—	Position of the "blepharoplast" (Kinetonucleus) varies and is not typical of Leishmania
		(<i>c</i>) Mouse	i.p.	"	+	—	—	—
12. <i>H. stratiomyiae</i>	Stratiomyas	Mice	per os	"	+	Free leishmania forms in organs	Died after 5 days	—

Summarising the results of Laveran and his collaborators, and Fantham and Porter, Laveran (1917) states that the experiments firmly establish the affinity between *Herpetomonas* (and *Crithidia*), on the one hand, and *Leishmania* on the other, an affinity which, from the phylogenetic point of view, is well recognised. Fantham (1915, 1915 *a*) goes a step further in his conclusions: he asserts that the experiments conducted by him and Porter, and Laveran and Franchini, prove that Leishmanias are arthropod-borne herpetomonias, and that these maladies have evolved mainly from insect flagellates. The experiments on artificial infection of vertebrates with these flagellates, according to this author, actually represent "leishmaniasis in the making."

Most of the flagellates with which Laveran and his collaborators dealt, and some with which Fantham and Porter worked, belong to insects which in nature are closely associated with some mammal, and it is known that some of these insects are capable of transmitting the haemo-flagellates to other vertebrate hosts, and infecting them, whilst others (*e.g.* the sand-fly) may eventually prove to be such intermediate hosts. On this account it is not surprising to find that the flagellates which parasitise the gut, and which may after all be insect stages of vertebrate haemo-flagellates, can produce infection when introduced into other vertebrates. In such cases no special adaptability is required.

Notwithstanding these facts, similar experiments repeated by other investigators produced negative results. Thus Chatton (1919) and Nöller (1920) repeated some of Laveran and Franchini's (1919) experiments with *H. ctenocephali*, but failed to find any traces of infection; similar experiments were made by Wenyon (1914) with the same flagellate, also with negative results. The last-named author also failed to produce any infection in rats inoculated with the flagellates of *Tabanus socius* (Wenyon, 1908).

As regards the pathogenicity of flagellates from insects which are not naturally associated with any vertebrate, this question is of more complex character. If such insect flagellates may become pathogenic to vertebrates when artificially introduced into them, we must assume that their power of adaptation is extraordinary, both as regards the time required for such adaptation in one generation, and as regards adaptation to the change of environment. This is the more surprising, if we take into account such protozoa as free-living amoebae which are frequently taken in by man and other animals, and pass through the whole digestive tract of the animal without becoming adapted to life in the latter, whereas closely allied forms of amoebae are found as obligatory parasites in the intestine of the same animal (*cf.* Wenyon, 1915; Dobell, 1919).

I shall now proceed with a description of my own experiments, and will return to the discussion of these questions later.

For my experiments I used the following flagellates: *Herpetomonas jaculum* from the water-scorpion (*Nepa cinerea*), *H. calliphorae* from the blue-bottle

fly (*Calliphora* sp.), and *Crithidia melophagia* from the sheep-ked (*Melophagus ovinus*). The animals experimented upon were mice, sticklebacks (*Gasterosteus aculeatus*), newts (*Molge vulgaris*) and frogs (*Rana temporaria*).

A. Experiments with Mice.

All the mice were inoculated intraperitoneally with the contents of the gut of insects infected with flagellates diluted with a physiological solution of sodium chloride. The animals were sacrificed after varying periods had elapsed, their blood being examined regularly till their death. After the animal was killed, cultures from its blood and organs—spleen and liver usually—in the NNN medium, and stained smears of the blood, peritoneal fluid, liver, spleen and, sometimes, bone-marrow and lungs were made. The blood and peritoneal fluid were also usually examined in the fresh condition.

I. Mice inoculated with *C. melophagia*.

Experiment 1. Mouse inoculated on October 19th, blood examined for two days with negative results. Sacrificed on October 21st. Stained smears of blood, peritoneal exudate, liver and spleen showed no parasites. Cultures made from the heart-blood, liver and spleen kept at 30° C. showed no parasites for 40 days.

Experiment 2. Mouse inoculated on October 19th. Blood examined with negative results till October 25th, when the animal was sacrificed. Stained smears (as preceding) showed no parasites. Cultures (as preceding) remained sterile for 36 days.

Experiment 3. Mouse inoculated on October 19th. Blood examined with negative results till October 29th, when the animal was sacrificed. Stained smears and cultures (as preceding) negative. Cultures examined for 32 days.

In experiments 1, 2 and 3 the three mice were inoculated with the contents of five “ked” guts; their condition was normal till death. The peritoneal fluid when examined showed numerous leucocytes.

Experiment 4. Mouse inoculated on July 15th. Blood examination during 25 days negative. Sacrificed August 9th. Stained smears of heart, blood, liver and spleen showed no parasites. Cultures from the blood and these organs kept at 24° C. remained sterile (27 days).

Experiment 5. Mouse inoculated on July 15th. Blood examination for 35 days negative. Sacrificed on August 19th. Stained smears and cultures (17 days) as in the preceding case—negative.

II. Mice inoculated with *H. calliphorae*.

Experiment 6. Mouse inoculated on August 9th. Sacrificed next day. Stained smears of blood, liver, spleen, kidney, bone-marrow, lung and peritoneal fluid were negative. Cultures from blood kept at 24° C. remained sterile (56 days).

Experiment 7. Mouse inoculated on August 9th. Sacrificed on the 19th. Stained smears (as preceding)—negative. Spleen culture showed no flagellates for eight days, when it became contaminated with bacteria.

In experiments 6 and 7 the peritoneal fluid showed numerous leucocytes.

III. Mice inoculated with *H. jaculum*.

Experiments 8 and 9. Two young mice inoculated on September 15th. Died next day. Peritoneal fluid and blood contained numerous bacteria. Blood cultures also showed bacteria.

Experiment 10. Young mouse inoculated on September 15th. Found dead on 17th. Not examined on account of decomposition.

Experiment 11. Mouse inoculated on October 26th. Sacrificed next day. Stained smears of blood, spleen and liver—negative. Peritoneal fluid showed numerous leucocytes. Blood cultures (at 30° C.) remained sterile for 11 days. The animal was in weak condition when killed.

Experiment 12. Young mouse inoculated on September 15th. Sacrificed on the 22nd. Blood examination till that date, negative. Smears of organs (as above)—negative. Blood cultures (13 days at 24° C.)—negative.

Experiment 13. Mouse inoculated on September 6th. Sacrificed on the 22nd. Blood examination till that date—negative. Smears of organs (as above)—negative. Blood cultures (13 days at 24° C.)—negative. For four days the mouse was in a weak condition, but recovered later.

Experiment 14. Mouse inoculated on October 26th. Sacrificed after one month (November 26th). Blood examination during that period revealed no parasites. Smears of blood, liver, spleen and peritoneal fluid—negative. Blood cultures kept at 24° C. remained sterile.

On the next day after inoculation the mouse was in weak condition, but soon recovered. It later developed gangrene on the tail, and examination *post mortem* showed a suppurative nodule in the liver, which, as I am told, is not uncommon in mice.

Experiment 15. Mouse inoculated on November 26th. After 3½ hours its blood and peritoneal fluid were examined fresh and stained. No parasites were found, but the exudate showed numerous leucocytes. The blood was regularly examined for 15 days, but showed no parasites. The mouse was sacrificed after 15 days; in smears of its organs no parasites could be found. Blood cultures kept at 24° C. remained sterile.

In the preceding experiments different doses of flagellates were inoculated into the mice. Nos. 8, 9, 10 and 12 received the contents of four *Nepa* guts each; Nos. 11 and 14—two each; No. 15—one gut.

As the experiments on mice show, there was no trace of flagellate infection in any of the cases recorded. Blood examinations were made at periods ranging from 3½ hours to 35 days with the same results. Cultures (at 24° C. and 30° C.) from the blood and organs of the infected animals examined in some cases for 56 days likewise failed to reveal any parasites.

B. Experiments with Fish.

I. *Sticklebacks* (*Gasterosteus aculeatus*) fed on the flagellates of *Nepa cinerea* (*H. jaculum*).

The contents of the gut of *Nepa* were examined for flagellates. If present, the gut was fed to the fish. It was always eaten immediately after being offered. Altogether 11 feeding experiments were conducted (each fish kept in a separate jar) varying according to the number of days they were fed, the number of *Nepa* guts they had eaten, and the length of time from the last meal to the date when they were sacrificed. For comparison, control fish were examined.

The examination of infected fish was conducted as follows. First the gill operculum was removed to expose the heart. Blood was then taken directly

from the heart either by means of a sterile pipette or by cutting the main artery (*Truncus arteriosus*) and allowing the blood from it to drop on a slide. Then, without injuring the alimentary canal (to avoid mixing its contents with other organs) the liver and spleen were removed and smeared on a slide. Films of the peritoneal fluid were also made. Lastly, the alimentary canal was totally removed and the stomach separated from intestine. The contents of the stomach and intestine, as well as the blood and peritoneal exudate, were examined both fresh in normal saline and in stained preparations.

Experiment 16 (VII). Fish fed on September 30th with one gut, October 1st, one gut, October 4th with two guts, one after another. Altogether it had four guts. *Killed two hours after last meal.* The insect gut was still undigested and nearly intact. The flagellates were quite motile in the stomach, but none were found in the intestine. The heart blood contained no flagellates, nor were any forms present in the liver, spleen and peritoneal fluid.

Experiment 17 (VIII). Fish fed on four guts (as preceding). *Killed $3\frac{3}{4}$ hours after last meal.* The insect gut was only partly digested. Motile flagellates were found in the stomach and a few already made their appearance in the intestine. No flagellates were found in the blood, peritoneal fluid, liver and spleen.

Experiment 18 (IX). Fish fed on three guts (as preceding, but only one gut on October 4th). *Killed five hours after last meal.* The insect gut was nearly digested. Few flagellates were found motile in the stomach, but none in the intestine. No forms found in the blood, exudate or liver and spleen.

Experiment 19 (IV). Fish fed on 12 guts (Sept. 17th one, Sept. 18th one, Sept. 20th two, Sept. 21st, 22nd, 23rd, 24th, 27th, 28th, 29th and 30th per one). *Killed five hours after last meal.* No visible traces of flagellates were found in the digestive tract, blood, exudate, liver and spleen.

Experiment 20 (III). Fish fed on nine guts (Sept. 17th, 18th, 20th, 21st per two, Sept. 22nd one). *Killed about five hours after last meal.* In the digestive tract flagellates were found with nuclei hardly visible, cytoplasm vacuolised, and shape of the body distorted. Apparently being digested. None were found in the blood, exudate and other organs.

Experiment 21 (XI). Fish fed on one gut (Sept. 6th). *Killed 18 hours after meal.* No live flagellates in fresh stomach contents. After the latter were stained, the flagellates were found to be apparently partly digested, their body being swollen, staining deeply and no inner structures being visible. There were no visible traces of the flagellates in the intestine. The insect gut was already wholly digested at this time. No forms of the flagellate were found in the blood, exudate and organs.

Experiment 22 (X). Fish fed on two guts containing an exceptionally large number of herpetomonads. *Killed 18 hours after the meal.* The insect gut was not yet quite digested and the alimentary canal contained some live flagellates, whilst others were found dead. None were found in the blood, exudate and organs.

Experiment 23 (I). Fish fed on ten guts (Sept. 16th two, Sept. 17th four, Sept. 18th, 20th per two). *Killed about 24 hours after last meal.* Insect gut wholly digested. No traces of flagellates in the digestive tract, blood and organs.

Experiment 24 (II). Fish fed on ten guts (Sept. 17th one, Sept. 20th two, Sept. 21st three, Sept. 22nd, 23rd per two). Results as in preceding.

Experiment 25 (VI). Fish fed on two guts (Sept. 17th and 18th). *Killed about 48 hours after last meal.* Results as in experiments 23 and 24.

Experiment 26 (V). Fish fed on seven guts (Sept. 17th, 18th, 20th, 21st, 22nd, 23rd, 24th per one). *Killed five hours after last meal.* The insect gut was nearly digested. In the

fresh contents of the stomach there were numerous dead flagellates, the bodies of which were distorted, the outlines and shape being very irregular and the cytoplasm vacuolised. In a stained preparation of the stomach contents in some of the flagellates the nucleus was still visible, in others it was diffused in the cytoplasm, and in some not visible at all. No flagellates were found in the blood of this fish, but smears of the spleen and liver contained several of them. This result was obviously due to carelessness on my part, as in this case the spleen and liver were separated from the intestine after the latter had already been removed from the fish. In this way the organs named were liable to be contaminated from the contents of the digestive tract. This is confirmed both by the fact that all the flagellates seen by me in the fresh contents of the fish-gut were already dead and had partly undergone digestion, and by the experiments adduced above in which the technique employed prevented such accidents from taking place.

In general, not only no harmful effect was observed from feeding the sticklebacks on the infected guts of *Nepa* for periods from one day to a fortnight, but they seemed to thrive on the food, being all the time very active and readily taking the guts whenever they were offered to them. Not one of the fish died from this food, and I may even say that the fish fed in this way were in better condition than the control fish kept in a tank with weeds and left to find food for themselves in the natural way.

These experiments show clearly that in the fish the flagellates are digested in the same way as any other food, and the fact that the flagellates remain alive for a considerable time only points to their vitality and power of resistance. This is not so surprising, taking into account that the flagellates are already adapted to life in the insect gut, the digestive juices of which do not differ materially from those in the higher animals.

These experiments also show that as long as any part of the insect gut remains undigested, thus protecting the flagellates enclosed in it from the action of the gastric juices, the flagellates in this part may remain alive. The action of the juices on the parasites is also exhibited in the fact that, whereas in the normal insect gut the flagellates are very numerous, very few can be

Table II.

Experiments on feeding sticklebacks on H. jaculum from Nepa cinerea.

No. of Experiment	Total No. of insect guts fed	No. of insect guts in last meal	Time from last meal to examination. Hours	Condition of food in the digestive tract of the fish (examined fresh)		Parasites in the blood, peritoneal fluid, liver and spleen of the fish
				Insect guts	Flagellates	
16	4	2	2	Undigested	Living	Absent
17	4	2	3 $\frac{3}{4}$	Partly digested	„	„
18	3	1	5	„	Few living	„
19	12	1	5	„	None found	„
20	9	1	5	„	Dead	„
21	1	1	18	Digested	„	„
22	2	2	18	Partly digested	Few living	„
23	10	2	24	Digested	None found	„
24	10	2	24	„	„	„
25	2	1	48	„	„	„

found in the digestive tract of the fish, although practically the entire contents were examined in each case. A glance at Table II will show the correlation existing between the progress of digestion in the fish and the condition of the flagellates in its digestive tract, both depending also on the number of insect guts eaten by the fish (apparently the number of guts taken with the last meal is of greater importance than the total number, as a comparison between experiments 21 and 22 shows).

II. *Sticklebacks inoculated with H. jaculum.*

Experiment 27 (XVI). Fish inoculated intraperitoneally found dead next day. Death due to mechanical injuries, for immediately after inoculation the fish was seen to float on its side (probably, swim-bladder injured). Peritoneal fluid examined fresh, showed numerous bacteria and several motile flagellates. Stained smears of blood, liver and spleen showed no forms of the flagellates.

Experiment 28 (XVIII). Fish inoculated intraperitoneally. Killed two days after inoculation. Peritoneal fluid showed plenty of motile flagellates; blood and organs negative. In the liver bacteria were visible.

Experiment 29 (XIX). Fish killed four days after intraperitoneal inoculation. The fish looked weak, its movements were slow. *Post mortem* showed signs of a haemorrhage on the genital glands, probably caused by injection. Fresh blood showed bacteria; in the peritoneal exudate numerous bacteria and one or two living flagellates were visible. The exudate also contained numerous leucocytes. Stained preparations of the blood, liver, spleen, revealed no traces of flagellates.

Experiment 30 (XVII). Fish killed six days after intraperitoneal inoculation. The fish looked quite normal before it was killed. In fresh peritoneal fluid bacteria were visible. No traces of the flagellates were found in the blood and organs.

Experiment 31 (XV). Fish inoculated subcutaneously died in half an hour after the operation, evidently from mechanical injuries (the bases of the fins and tail immediately became white, probably from penetration of air introduced by injection). An examination of the fluid from under the skin showed motile flagellates.

Experiment 32 (XIV). Killed 24 hours after subcutaneous inoculation. A slight swelling appeared on one side of the body, but disappeared when dissected. The fluid taken from under the skin showed no parasites, but it contained numerous leucocytes and bacteria. The liver and spleen also showed only a few bacteria, and no flagellates were found in the blood.

Experiment 33 (XIII). Killed two days after subcutaneous inoculation. Fresh fluid from under the skin showed no flagellates, but several of them were recovered from the peritoneal fluid, which contained numerous leucocytes, some of which had phagocytised flagellates enclosed in them, together with bacteria.

Blood and spleen negative; in the liver bacteria were visible.

Experiment 34 (XII). The fish was left alive after subcutaneous inoculation and seemed to be quite normal for three weeks, after which it was placed in a tank with other fish.

The experiments on inoculation of sticklebacks with *H. jaculum* show that no infection whatever is produced by the flagellates thus introduced into the fish. The most careful examination of the body fluids and organs did not reveal the presence of any forms of the parasites, except in the site of inoculation, or of any lesions produced by them, whereas the bacteria naturally occurring in *Nepa* and introduced into the fish together with the flagellates, seem to find

a footing there, being nearly always present either free or in leucocytes in the blood, peritoneal exudate and organs. These experiments have, however, again shown the extent to which the insect flagellates are capable of retaining their vitality in the body of the fish under conditions unusual to them. It is seen that they can be found alive in the peritoneal cavity and subcutaneous fluid of the fish for two to four days, but they disappear altogether on the sixth day, being probably phagocytised, as on several occasions I was able to find leucocytes that contained flagellates in degenerate condition within them.

A summary of these experiments is given in Table III.

Table III.

Experiments on inoculation of sticklebacks with H. jaculum.

No. of experiment	Mode of inoculation	Date of inoculation	Interval between inoculation and examination	Results of examination of fresh blood and body fluids	Results of examination of stained smears	Remarks
27	i.p.	28 x. 20	24 hrs (died)	In perit. fluid <i>motile flagellates</i> and bacteria. Blood—negative	Blood, liver and spleen—negative	Death due to mechanical injuries
28	—	—	2 days (killed)	In perit. fluid <i>motile flagellates</i> . Blood—negative	Blood, liver and spleen—negative. Liver contained bacteria	—
29	—	—	4 days (killed)	In perit. fluid <i>single flagellates</i> , numerous bacteria. Blood—negative	Blood, liver and spleen—negative. Perit. fluid contained numerous leucocytes	Fish looked weak. <i>Post mortem</i> genitalia showed signs of hæmorrhage. (Mechanical injury)
30	—	—	6 days (killed)	In perit. fluid <i>no flagellates</i> , numerous bacteria. Blood—neg.	Negative	Fish quite normal
31	s.c.	26 x. 20	30 min. (died)	Subcutaneous fluid contained <i>motile flagellates</i>	—	Died from penetration of air introduced by injection
32	—	—	24 hrs (killed)	In subcutaneous fluid <i>no flagellates</i> ; numerous leucocytes and bacteria	Negative. Spleen and Liver contained bacteria	Slight swelling on one side of the body
33	—	—	2 days (killed)	In subcut. fluid <i>no flagellates</i> . In perit. fluid several <i>flagellates</i> free and phagocytized, and bacteria	Negative. Liver contained bacteria	—
34	—	—	—	—	—	Remained quite normal for 3 weeks, when it was transferred to a tank with other fish

C. Experiments with Amphibians.

I. *Newts* (*Molge vulgaris*) inoculated with, and fed on, *H. jaculum*.

(a) *Newts* inoculated intraperitoneally. November 11th.

Experiment 35. Newt killed after 24 hours. No forms of the flagellates found in fresh peritoneal fluid and blood, nor in stained smears of the same, and liver and spleen.

Experiment 36. Newt killed after two days. Results as above. Peritoneal fluid contained numerous leucocytes.

Experiment 37. Newt killed after four days. Results as preceding.

Experiment 38. Newt killed after five days. Results as preceding.

Experiment 39. Newt was left alive.

(b) Newts fed on *H. jaculum* introduced by a pipette *per os*.

Experiment 40. Newt killed after 24 hours. Examined contents of the alimentary tract, blood, liver, spleen, peritoneal fluid. Nowhere were any traces of the flagellates visible.

Experiment 41. Newt left alive.

The experiments on newts show that in their organism the flagellates are apparently very soon disposed of, no traces of them being visible even after 24 hours. There was no change in their condition, and two newts inoculated intraperitoneally and *per os* respectively remained living, showing no signs of infection.

II. *Frogs (Rana temporaria) inoculated with H. jaculum.*

Experiment 42. Young frog inoculated into the dorsal lymph-sinus on November 26th. Killed after 24 hours. Examination of fresh blood and fluid from the lymph-sinus, and of stained blood, sinus fluid, liver and spleen revealed no traces of parasites.

Experiment 43. Young frog inoculated on the same day intraperitoneally. Results as in preceding.

Experiment 44. Adult ♀ frog inoculated into the dorsal lymph-sinus on the same day. Killed after 19 days. Blood examination during the period preceding death—negative. *Post mortem* results as in Nos. 42 and 43. Blood-cultures from this frog kept at 24° C. showed no parasites for more than a month.

The experiments with frogs thus also produced negative results, although the mode of inoculation through the lymph-sinus in two cases afforded an easy and natural point of access to the blood-stream of the frog.

III. OBSERVATIONS ON THE VITALITY OF SOME INSECT FLAGELLATES.

The degree of vitality of some flagellates when exposed to abnormal conditions is shown in some of the experiments already recorded. As these experiments have demonstrated, the flagellates can remain alive in the intestine of fish for five hours, and in some cases (experiment 22) for 18 hours, resisting the action of the digestive juices. When injected subcutaneously or intraperitoneally into fish, they may be found alive in the peritoneal fluid for four days. I have also kept insect flagellates living in sealed drops with a physiological solution of sodium chloride and with sterile nutritive bouillon at different temperatures. The results were as follows:

Contents of the gut of the sheep-ked (*Melophagus ovinus*) infected with *Crithidia melophagia* diluted with normal saline were kept at room temperature and at 30° C. In the portion that was placed at 30° C. the flagellates lived only one day, but those that were kept at room temperature lived seven days. No multiplication was observed, and the flagellates gradually lost their motility.

Contents of the gut of the water-scorpion (*Nepa cinerea*) infected with *Herpetomonas jaculum* were placed at room temperature in saline and in slightly alkaline nutritive bouillon. In both cases the flagellates remained alive for five days. It was observed that towards the end of that period their movements became slower, many had become rounded off, retaining their flagella, others had swellings on different parts of their body.

Regarding the vitality of *C. melophagia* Flu (1908) mentions that it is possible to keep them alive in normal saline only for two hours, and in serum for eight hours at room temperature. In an ice chest they remained alive for six days. According to Porter (1909) the same flagellates remain alive for several hours at room temperature. Georgéwitch (1910) kept his flagellates living in drops of serum for several days.

CONCLUSIONS.

In my experiments on artificial infection of mice with *Crithidia melophagia*, *Herpetomonas jaculum* and *H. calliphorae*, I was unable to confirm the results obtained by Laveran and his collaborators, having found no traces of infection or of the presence of any forms of flagellates in the animals experimented upon. Whether this is due to difference in methods or to some other cause, it is difficult to tell. The most remarkable feature of the experiments of these observers is that positive results were produced so invariably, whereas in my experiments made on fifteen mice which were most carefully examined at different periods of their life and *post mortem*, and in all cases tested by cultural methods, the results were always negative. This difference cannot be due to the authors mentioned having used flagellate cultures for inoculation, as they started experimenting with cultures only since 1919; up to that time they produced positive results by inoculating flagellates directly from the guts of the insects and otherwise. As it is, my experiments, which are not so numerous and varied as those discussed, although not disproving the experiments described by Laveran and his collaborators, at any rate clearly show, together with the experiments of Wenyon (1908, 1914), Chatton (1919) and Nöller (1920), that artificial infection with insect flagellates requires further study and the results already recorded do not permit as yet of forming general conclusions as to the pathogenicity of insect flagellates, and their relation to Leishmaniasis.

In connection with this, I should like to add that in some of the experiments discussed the results are difficult to understand. For instance, Franchini and Mantovani's (1915) experiments with *H. muscae-domesticae* are quite incomprehensible. The authors state that blood cultures from a rat inoculated with these flagellates produced only "anaplasma" bodies. These bodies, when re-inoculated into a mouse, gave rise to leishmania forms. The "anaplasma," as we know it, is merely a structureless granule within red cells, which stains red with Romanowsky stains, and it is still questionable whether it is in reality an organism at all, although some recent workers definitely assert that they

are protozoa (cf. Lignières, 1919; Di Domizio, 1919). Whatever the case may be, there are no grounds for connecting the "anaplasma" with the flagellates.

In the experiments of these authors and Laveran and his colleagues reference is frequently made to leishmania forms with only a single nucleus. It must be very doubtful if these can be regarded as such. In the examination of smears of organs, in experiments of this kind, only undoubted leishmania forms should be considered. According to these authors, leishmania forms are also produced by infecting mice with *C. melophagia* (Laveran and Franchini, 1914 b, 1919 a). As Nöller's (1919, 1919 a, 1920) observations have proved this flagellate to be a trypanosome (*T. melophagium*), its inoculation into mice would be likely to produce in the latter trypanosomes, and not leishmanias.

The experiments of Fantham and Porter (1915, 1915 a) are of especial interest in this respect. These authors have made experiments on artificial infection working chiefly with flagellates of insects not associated normally with any vertebrate animal. They claim to have proved that insect flagellates may be successfully inoculated into and fed to different warm- and cold-blooded animals. The flagellates become pathogenic to these animals and produce in them symptoms resembling Kala-azar and other leishmaniases.

Fantham (1915, 1915 a) concludes from these experiments that "the occurrence of natural herpetomonads in invertebrates must not only be acknowledged, but it must be allowed that they may become pathogenic, when introduced into vertebrates," thus apparently extending the results of the experiments discussed to all insect herpetomonads.

I have repeated some of the experiments recorded by Fantham and Porter with the negative results already described. In the case of infection of fish, my experiments were much more numerous and varied than those recorded by the authors named. In inoculating frogs, the site of injection (lymph-sinuses) would seem to afford the easiest point of access for the flagellates into the blood stream of the host. Notwithstanding these facts, I have utterly failed in producing even the slightest sign of infection in these animals, and have never found any forms of the flagellates introduced into them which would suggest infection.

The conclusions of Fantham (1915, 1915 a) already quoted, and the more general conclusion that leishmaniases are "arthropod-borne herpetomoniasis" are very interesting from the theoretical point of view, and it is quite possible that later discoveries will prove this to be a fact, and not merely a hypothesis.

The facts available at present, however, do not in my opinion permit one to assert that the natural herpetomonads in insects, especially in those not associated with vertebrates, may become pathogenic, when introduced into the latter, as the author suggests.

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AN ANNOTATED LIST OF THE ANIMAL PARASITES OF FOXES¹.

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FEW people have any conception of the rapid growth and the importance of the new industry of raising black and silver foxes for their fur. According to Dearborn, 1917, the first profitable fox ranch was established in the Canadian Province of Prince Edward Island in 1894. This ranch, the forerunner of a remarkable industry, was stocked with two pairs of silver foxes, a rare and beautiful colour phase of the common red fox found in nearly all of the United States and Canada.

Until 1910, the methods of growing of these foxes was kept a profound secret and practically "monopolised" by a few Prince Edward Islanders. Now there are many farms throughout Canada and the Northern United States and some foreign countries, notably Japan. Literally millions of dollars are invested, and prime breeding stock sells for many times the value of a high grade horse. In spite of the very heavy decline in prices of furs, prime pelts of the silver foxes are to-day worth from \$500 to \$1200 each.

Until very recently practically no attention from an economic view-point has been paid to the animal parasites of foxes. They have been regarded as of merely general zoological interest, and that mainly from the systematic side. Now, with the rapid development of the fur farming industry, this condition is rapidly changing, for it is clear that intensive studies of any factors affecting the health or the quality of the fur of foxes are of great economic importance.

Data regarding these points are not easy to obtain. This is in part due to the widespread ignorance regarding the nature of parasitic diseases. In part it is due to the fact that to-day the average grower of silver foxes depends for his largest profits on the sale of breeding stock and hence is loath to admit that he has any trouble from disease. Indeed, it is commonly stated that foxes are remarkably free from disease. However true this may be of the animals in nature, there is no reason to hope that the condition will long prevail among the domesticated forms.

In general it might be supposed that foxes would be subject to the same parasites as are dogs and other Canidae. How true this is we have not at present sufficient data to judge. Certain it is that many parasites which infest

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dogs have not yet been reported for foxes. On the other hand, several fox parasites are thought to occur exclusively in these animals.

In the following list are included such animal parasites as have been reported for foxes, together with notes on their occurrence, and probable pathogenic significance.

PROTOZOA.

There are very few records of the occurrence of parasitic protozoa in foxes. While several serious protozoal diseases are known to occur in dogs, they are fortunately prevalent chiefly in the tropics and in sub-tropical regions. Successful fox fur farming is limited to the cooler zones, a long cold season being an important factor in producing suitable fur growth.

Trypanosoma brucei Plimmer and Bradford, 1879. This trypanosome, the well-known causative organism of the tsetse-fly disease "Nagana" of cattle has been experimentally transmitted to the fox by Yakimoff (1917). *Trypanosoma gambiense* has also been inoculated successfully into experimental foxes by Laveran (1915).

In spite of the extensive study devoted to the trypanosomes, there does not seem to be any record of their occurrence in the fox in nature.

Trichomonas sp.—Dr W. L. Chandler informs me that he has found in Michigan a species of *Trichomonas* occasional in foxes. While it is the usual tendency to regard the members of this genus as harmless commensals, recent studies, notably those of Hadley (1916) and Tyzzer (1920), indicate that under some conditions they may be highly pathogenic.

Isospora bigmenina (Stiles, 1891). (Synonyms: *Coccidium bigeminum*, Stiles, 1891; *Diplospora bigemina* (Stiles) Wasielewski, 1904.) In foxes from three separate ranches we have found a coccidium which agrees fairly closely with the species found by Stiles in the dog. The rounded somewhat ovoid cysts have a double-contoured wall and measure on the average 30μ by 25μ . Both single and two-spored oocysts are found in the fresh faeces.

Weidman (1915) has discussed the finding of what appears to be this same coccidium in the faeces of two swift foxes from the Western United States, which were kept in the Philadelphia Zoological Gardens. "Both foxes when in isolation showed diarrhoea, the one more marked than the other. In the former the oocysts were in great numbers and in about a week the animal died. The second fox, on the other hand, passed very small numbers of cysts, spontaneously recovered, and after isolation for several weeks was returned to exhibition, the faeces no longer containing oocysts." Autopsy of the dead fox disclosed a most marked haemorrhagic and ulcerative enteritis involving both small and large parts of the bowel. Within the ulcers were found mature naked protozoa, and upon the mucosal surface double-walled oocysts corresponding to those found in the faeces.

As the oocysts found in the fox averaged considerably larger than those reported by Stiles for the dog Weidman considered them sufficient to constitute a new variety, *canivelocis*. The organism should be studied in greater detail.

in order to determine definitely its relation to the species described by Stiles.

Theileria sp. Yakimoff and his associates have reported (1917) the finding of an undetermined species of *Theileria* in the blood of foxes in Russian Turkestan.

CESTODA.

Some thirty species of cestodes have been reported as occurring in foxes. When the synonymy is considered this list is reduced to less than half. In none of the cases, in so far as I have found, is there any reference to the effects upon the fox. The pathogenicity of some, the species which affect other hosts than the fox, is well known.

The species reported for the fox are listed alphabetically below, together with notes on synonymy and on the host species where available.

Bothriocephalus similis Krabbe, 1865.

In *Vulpes lagopus*, Greenland.

Cysticercus vulpis (Gmel. 1790) Zed. 1803.

(Synonym: *Taenia vulpis* Gmel. 1790.)

In *Vulpes*, Europe.

Diphyllbothrium latum (Linn. 1758) Luehe, 1910.

(Synonyms: *Taenia lata* Linn. 1758; *Bothriocephalus latus* Bremser, 1819; *Dibothriocephalus latus* Luehe, 1899.)

Occurs rarely in the common fox of Europe (Braun; Railliet).

Dithyridium elongatum (Blumberg, 1882) Raill. 1893.

(Synonyms: *Cysticercus elongatus* Blumberg, 1882; *Cysticercus bailleti* Railliet, 1885.)

This larval form, resembling a plerocercoid except that it possesses four suckers, occurs in the peritoneal or pleural cavity, free or encysted in dogs and cats. Neumann (1896) reports finding a great number in the peritoneal and pleural cavities, as well as beneath the serous membrane of the liver and lung of a fox. Allesandri (1907) also reports it for the fox.

Neumann presents evidence indicating that these forms may be erratic larvae of *Mesocetoides lineatus*, which he commonly found in the intestines of the same animals.

Mesocetoides canis-lagopodis (Rud. 1810) Luehe 1894.

(Synonyms: *Taenia canis-lagopodis* Rud. 1810; *Ptychophysa canis-lagopodis* Loennb. 1896.)

From *Vulpes lagopus*.

Mesocetoides lineatus (Goeze, 1782) Braun, 1898.

(Synonyms: *Taenia lineata* Goeze, 1782; *Taenia pseudo-cucumerina* Bailliet, 1863; *Ptychophysa lineata* Hamann, 1885.)

Various writers report this as from the common fox of Europe. Some of these records relate to the following species, *M. litteratus* Batsch.

Mesocetoides litteratus (Batsch, 1786) Dolley, 1894.

(Synonyms: *Taenia litterata* Batsch, 1786.)

Type host, European fox.

Monodoridium utriculifera Walter, 1866.

(Synonyms: *Taenia utriculifera* (Walters, 1866) Linstow, 1878; according to Stossich, 1896 = *Mesocetoides litteratus*.)

Type host *Vulpes vulpes*.

Multiceps multiceps (Leske, 1780) Hall, 1910.

(Synonyms: *Taenia coenurus* Kuchenmeister, 1853; *Cystotaenia coenurus* Leuckart, 1863; *et al.* For details of the extensive synonymy see Hall, 1910.)

Various writers have reported the adults of this species for the blue fox, *Vulpes lagopus*, and for the red fox *Vulpes vulpes*. While Hall questions all of these records there is no apparent reason for so doing.

Taenia crassiceps Rud. 1819.

Type host a fish, *Gadus merluccius*. The record for the fox, by Olt and Strase, 1914, is due to confusion with *Taenia crassiceps* Zeder.

Taenia crassiceps (Zeder, 1800) Rud. 1810.

(Synonyms: *Alyselminthus crassiceps* Zeder, 1800.)

Type host *Vulpes vulpes*, Europe, "Also reported for *Vulpes melanogaster*" (Stiles and Hassall).

Taenia hyperborea Linstow, 1905.

Vulpes lagopus, in E. Greenland.

Taenia ovata Molin, 1858.

Vulpes vulpes, and *Vulpes lagopus*.

Taenia pisiformis (Bloch, 1780) Gmel. 1790.

(Synonym: *Taenia serrata* of various authors, but not as used by Goeze, 1782.)

Reported by Cobbold (1786) and various other writers for common fox of Europe.

Taenia polyacantha Leuckart, 1856.

Vulpes vulpes, Germany.

Tetrabothrius vulpis Blainv. 1828.

Type host, fox.

TREMATODA FROM FOXES.

Ascocotyle longa Ransom, 1920.

Intestine of *Vulpes lagopus*, National Zoological Park, Washington, D.C.

Parasites of Foxes

Ascocotyle nana Ransom, 1920.

Same data as for *A. longa*.

Conchosomum alatum (Goeze, 1782) Railliet, 1896.

(Synonyms: *Alaria vulpis* Schrank, 1788; *Festucaria alata* Schrank, 1790; *Fasciola alata* Rud. 1793; *Distoma alatum* Zed., 1800; *Holostomum alatum* Nitzsch, 1819; *Hemistomum alatum* Dies, 1850; *Diplostomum alatum* Parona, 1894.)

According to Olsson (1876) from *Vulpes alopax* and *V. lagopus*.

Reported for various hosts in Europe, Egypt, Brazil and New South Wales.

Cotylophallus venustus Ransom, 1920.

Intestine of Alaskan fox, *Vulpes lagopus*, National Zoological Park, Washington, D.C.

Echinostoma trigonocephalum (Rud. 1803) Cobbold, 1860.

(Synonyms: *Fasciola trigonocephala* Rud. 1802; *Distoma trigonocephalum* Rud. 1809.)

Reported by Stossich (1892) for *Vulpes vulpes*, intestine.

Heterophyes heterophyes (Siebold, 1852) Stiles and Hassall, 1900.

(Synonym: *Coenogonimum heterophyes*.)

Looss (1902) records it doubtfully from fox.

Metorchis conjunctus (Cobbold, 1860) Looss, 1899.

(Synonym: *Distomum conjunctum* Cobbold, 1860.)

"Numerous examples in the liver of the American red fox, *Canis fulvus*"—Cobbold (1860).

Opisthorchis felineus (Rivolta, 1884) Blanchard, 1895.

(Synonym: *Distomum conus* Gurlt, 1831, nec Creplin, 1825.)

Gall ducts of fox, as well as of dogs, cats and man.

Opisthorchis noverca Braun, 1902.

Not found in foxes. Statements as to its occurrence are based on McConnell's erroneous assumption that his specimens from man in India were identical with Cobbold's *Distomum conjunctum*.

Pseudamphistomum truncatum (Rud. 1819) Luehe, 1909.

(Synonyms: *Amphistoma truncatum* Rud. 1819; *Distomum conus* Creplin, 1825; *Metorchis truncatus* Looss, 1899.)

In gall ducts of fox, dog, cat, seals, etc.

NEMATODA.

Ancylostoma caninum (Ercolani, 1859) Looss.

(Synonyms: *Strongylus trigonocephalus* and *S. tetragonocephalus* Rud. 1808; *Dochmius balsamoi* Parona and Grassi, 1877; *Uncinaria trigonocephala* Raill. 1885; *Ankylostomum tubaeforme* Linstow, 1885.)

There are numerous European records of the occurrence of this hookworm in foxes, but I do not know of any explicit American record. The adult hookworms from the fox which I have at hand are all of *Uncinaria polaris*, but I am not warranted in concluding definitely that all of the hookworm eggs which I have noted in faeces examinations are of this species.

Ancylostoma caninum is common in cats and dogs in this region. Hall reports it in 23 out of 67 dogs, or 34 per cent. at Detroit, Michigan. My records for Minnesota dogs are not extensive enough to justify a statement of percentages, I have found it in ten out of 30 cats examined. It is a significant fact that it is a common practice of fox growers to use cats as foster mothers of fox puppies if the vixen is unable to provide for them.

Belascaris marginata (Rud. 1802) Leiper, 1907.

(Synonyms: *Ascaris marginata* Rud. 1802 *pro parte*.)

This common ascarid of dogs frequently occurs in foxes and is the cause of serious trouble in the case of the pups. By most growers it is considered the most common, or practically the only "worm" of foxes, owing doubtless to the fact that the worms are to be found in the droppings. My examinations would lead me to think it less frequent than are hookworms. The latter were found in 26 out of 30 samples from four different farms, while ascarids were present in but six.

Belascaris vulpis (Frölich, 1789) Railliet and Henry, 1911.

(Synonyms: *Ascaris vulpis* Frölich, 1789; *Ascaris triquetra* Schrank, 1790.)

Railliet and Henry (1911) recognise the *Ascaris vulpis* of Frölich from *Vulpes vulpes*, as a valid species closely related to *Belascaris marginata*. The spicules are of the same length as in the latter species, but the caudal extremity of the body is excavated in a gutter-like manner (*creusée en gouttière*) and almost triangular with caudal wings well developed.

It is to be noted that among 200 ascarids from 25 Massachusetts dogs, Walton (1916) found two which he regarded as *Ascaris triquetra* Schrank (= *B. vulpis*).

Crenosoma semiarmatum Molin, 1861.

(Synonyms: *Strongylus decoratus* Creplin, 1847; *Liarhynchus vulpis* Dujardin, 1845.)

Reported by Bremser, Dujardin and Creplin, as found in the lungs and trachea of *Vulpes vulpes*.

Diectophyme renale (Goeze, 1782) Stiles, 1901.

(Synonyms: *Ascaris visceralis* Gmelin, 1789; *Strongylus gigas* Rud., 1802; *Eustrongylus gigas* Diesing, 1851.)

The giant kidney-worm was reported for both the European and the common American fox, by Rudolphi, 1819.

Eucoleus aerophilum (Creplin, 1839) Duj. 1845.

(Synonym: *Trichosoma aerophilus* Creplin, 1839.)

Trachea of *Vulpes vulpes*.

Filaria vulpis Comper.

Rudolphi questions whether this worm, reported by P. Comper as from the abdomen of a fox, was not merely a wandering ascarid from the intestine.

Habronema grimaldiae Seurat, 1915.

From stomach and intestine of the Algerian fox, *V. vulpes atlantica*.

Ollalanus tricuspis Leuckart, 1865.

From the tunic of the stomach of the cat; reported also for the fox.

Rictularia affinis Jägerskiöld.

Type specimens from the Egyptian fox; Seurat (1915) reports it for the Algerian fox, *V. vulpes atlantica*.

Spirocerca subaequalis Seurat, 1915.

From stomach of Algerian fox.

Spiroptera sanguinolenta Rud. 1819.

(Synonyms: *Strongylus lupi* Rud. 1809; *Filaria sanguinolenta* Schneider, 1866.)

In tumours in the stomach and oesophagus of various Canidae, including foxes. Embryos in the blood.

Spirura gastrophila (Mueller, 1895).

Seurat (1918) found this species in the Algerian fox.

Strongyloides longus (Grassi, 1885).

Intestine of fox, Russia. Romanovic (1914).

Strongylus vulpis Rud. 1819.

Bremser reported finding in the mesenteric glands of the fox a worm which Rudolphi lists with others under the heading "Entozoa vel Generis dubii, vel fictitia." It remains unknown. Obviously it cannot belong to the genus *Strongylus* as now delimited.

Trichinella spiralis (Owen, 1835) Railliet, 1895.

The fox is susceptible to infestation by *Trichinella spiralis*. Grüner (1916) describes trichinosis in captive wild animals including *Vulpes lagopus*.

Trichocephalus depressiusculus Rud. 1809.

(Synonyms: *Trichocephalus vulpis* Frölich, 1789; *Mastigodes vulpis* Zeder, 1803.)

I have found this in 12 per cent. of the foxes examined. It is reported frequently in the literature as occurring in these hosts.

Trichosoma plica Rud. 1819.

(Synonym: *Calodium plica* Duj. 1845.)

In the bladder of *Vulpes vulpes*.

Uncinaria criniformis (Rud. 1809) Railliet, 1899.

(Synonyms: *Ascaris criniformis* Goeze, 1782; *Strongylus criniformis* Rud. 1809; *Uncinaria stenocephala* Raill. 1884.)

Commonly found in European foxes and other Canidae. Also found in the Arctic fox *V. lagopus*.

Uncinaria polaris Looss, 1911.

Type host *Vulpes lagopus* from the National Zoological Gardens, Washington, D.C. Stiles (1902), who sent the material to Looss, states that it was the cause of an outbreak of uncinariasis among the Arctic foxes of the Zoological Garden.

Riley and Fitch (1921) have reported this species as very common in three out of four silver fox ranches examined, and as the undoubted cause of loss, not only through death of young stock, but through affecting the quality of the fur of infested animals.

Uncinaria vulpis Frölich, 1789.

Though the genus *Uncinaria* is now generally accepted, this, the type species, is listed by Looss as a *species inquirenda*.

LINGUATULINA.

Linguatula serrata Frölich, 1789.

(Synonyms: *Taenia rhinaria* Pilger, 1802; *Pentastoma taenoides* Rud. 1819.)

The adult of this problematic arthropod occurs in the nasal cavities of the fox, as well as of the dog, wolf, and a variety of animals.

ACARINA.

Demodex folliculorum (Simon, 1842).

This mite, the cause of the exceedingly stubborn follicular mange of dogs is known to occur, though rarely, in the fox. Apparently Gros (1845) was the first to definitely list the fox as a host. Since his time there have been a number of references, but nothing specific in the literature.

Sarcoptes scabiei vulpis (Fürstenberg, 1861) Raill.

Sarcoptic mange is widely distributed among foxes and fox farmers regard it as one of the parasitic diseases that are most to be feared because of its high degree of contagiousness. Since the disease was discovered, a thorough-going quarantine and inspection of all imported foxes has been maintained.

Fürstenberg and also Braun have called attention to the fact that the variety occurring in the fox is transmissible to man. Weydemann (1897) records a case where a fur dealer and his entire family became infested from a

mangey weasel skin which was subsequently found to harbour great numbers of what he identified as *Sarcoptes vulpis*.

Dermanyssus gallinae, the common red mite of poultry is said by Wood (1917) to be conveyed by foxes, skunks, weasels, etc.

IXODOIDEA.

Many species of ticks occur on a variety of hosts. The following species have been taken from the fox:

Amblyomma americanum (Linn. 1758).

Amblyomma tuberculatum Marx.

Dermacentor variabilis (Say, 1821).

Haemaphysalis bispinosa intermedia Warburton and Nuttall, 1909.

Haemaphysalis cinnabarina punctata (Canestrini and Fanzago, 1877).

Haemaphysalis inermis Birula, 1895.

Haemaphysalis leachi (Audouin, 1827) Neumann, 1897.

Ixodes canisuga Johnston, 1849.

Ixodes hexagonus Leach, 1815.

Ixodes marxi Banks, 1908.

Ixodes ricinus (Linnaeus, 1746 and 1758) Latreille, 1804.

Rhipicephalus sanguineus (Latreille, 1804).

MALLOPHAGA.

Trichodectes quadraticeps Chapman, 1887, has as its type host the California gray fox, *Urocyon cinereoargenteus*.

Trichodectes vulpis Denny, 1842 (Synonym: *T. micropus* Giebel, 1874) is from the common European fox.

SIPHONAPTERA.

Pulex irritans Linnaeus, 1858. *Vulpes* sp.

Pulex pallidus Taschenberg, 1880. *Vulpes niloticus*.

Ctenocephalus canis (Curtis, 1826) Baker, 1904. *Vulpes lagopus*, *V. vulpes*, *Urocyon cinereoargenteus*.

Ceratophyllus melis Curtis, 1832, "sur le Renard" Raill.

Chaetopsylla vulpes (Motscherlsky, 1840) Baker, 1905. *Vulpes vulpes*.

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NOTE.

Since the above paper was in print I have received two important publications bearing upon the topic of animal parasites in foxes.

The long awaited part of the *Index Catalogue of Medical and Veterinary Zoology* which deals with round worms is now available as Bulletin No. 114 of the Hygienic Laboratory of the U.S. Public Health Service. This will prove an invaluable aid to students of the Nematoda.

A paper by M. C. Hall, 1920, on "Intestinal Parasites found in Eighteen Alaskan Foxes" (*North American Veterinarian*, i. 123-124) reports on the examination of eighteen foxes from Saint George Island, Alaska. "Of the eighteen foxes, eight were infested with ascarids, ten with *Mesocestoides*, and one with *Taenia*, and one fox had a number of dipterous larvae, probably from fly-blown flesh. Some of the ascarids have been examined and found to be *Toxascaris*, possibly *T. limbata*, and all of them may belong to the same species. The infestations were not heavy, the largest number being twelve."

A surprising feature of the findings is the absence of hookworms. "This suggests that the island is free from this worm which is a serious pest to foxes elsewhere."



FRANCESCO REDI
1626—1697



CAROLUS LINNAEUS
1707—1778



CARL DE GEER

1720—1778

ON THE LIFE-HISTORY OF *HELICOSPORIDIUM PARASITICUM*, N.G., N.SP., A NEW TYPE OF PRO-
TIST PARASITIC IN THE LARVA OF *DASYHELEA OBSCURA* WINN. (DIPTERA, CERATOPOGONIDAE)
AND IN SOME OTHER ARTHROPODS.

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(With Plates IV—VI and 5 Text-figs.)

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I. PRINCIPAL HOST OF *HELICOSPORIDIUM* AND ITS INFECTION.

THE usual host of this new parasite, *Helicosporidium parasiticum*, is the larva of a Ceratopogonid, *Dasyhelea obscura* Winnertz, which lives in the decomposed sap filling the wounds of trees—elm and horse-chestnut. All the material used for this study was derived from the wounds of two trees only: (1) an elm tree standing on the Caius College ground at Newnham, facing Church Rate

Walk, and (2) a horse-chestnut standing between the School of Agriculture and Downing College.

In addition to the new parasite, the larvae of *Dasyhelea* harbour several other parasites, two of which—(1) a gregarine, *Allantocystis dasyhelei* Keilin, and (2) a parasitic yeast *Monosporella unicuspidata* Keilin—have already been dealt with in my previous papers (1920 *a* and *b*).

To find the larvae parasitised with *Helicosporidium*, one proceeds in the following manner: to a small quantity of the semi-fluid exudate collected from the wounds of trees, is added ordinary tap water sufficient to cover the bottom of a wide dish. The mixture is then thoroughly stirred and is left for an hour or more to settle. The numerous larvae of various sizes found at the bottom of the dish are then transferred by means of a pipette to a dish containing clean water and standing on a black or dark coloured surface. On careful examination of the contents of this dish it is noticeable that while the normal *Dasyhelea* larvae present a whitish but translucent appearance, a few individuals are usually seen which are white and opaque. Isolated on a slide and examined under the microscope, all these opaque larvae are found to be parasitised either with *Monosporella*, or with the new parasite *Helicosporidium*, or very exceptionally with both organisms together. As the proportion of larvae parasitised with *Monosporella* is very small, almost all the opaque and milky specimens of *Dasyhelea* larvae are found to be infected with the new parasite.

If we examine under the microscope a parasitised larva, compressed between slide and coverslip, sufficient to prevent movement, we see that its entire body cavity is filled with small round corpuscles, 5 or 6 μ in diameter. These corpuscles occupy all the spaces between the organs of the larva and extend through all the segments including the head.

Being free in the body cavity of the larva, the parasitic corpuscles are always seen circulating or even gushing from one segment to another. This movement is purely passive and is produced either by the contraction of the segments of the host, by the more or less regular movement of its internal organs, or by the rhythmic contractions of the heart and the consequent plasma circulation. These passive movements of *Helicosporidium* are very easily seen in the head of the larva, where the parasites are less densely crowded on account of the restricted spaces between the strongly developed muscles of the mouth-parts. The post-abdominal segments on the contrary are filled to such an extent with the parasitic corpuscles that these form a solid mass occupying the whole cavity of these segments. The posterior portion of the larva becomes thus very turgid, loses its mobility, and becomes very fragile. By pricking such parasitised larva with a fine needle or even by gentle pressure on the coverslip a milky fluid gushes from the wound, and this fluid when examined with the microscope is seen to be a pure suspension of parasitic corpuscles (Pl. V, figs. 1, 2 and 3).

All the larvae which were recognised from their external appearance as being infected, had already arrived at such an advanced stage of infection, that

no stages of the multiplication of the parasite could be detected. To find the earlier stages of infection it was impossible to select the parasitised larvae with the naked eye. For this purpose each larva had to be examined separately under a high magnification, a very long and difficult task, because (1) the larvae are insufficiently transparent owing to their cuticle being lined with a layer of the fat body, whilst (2) the early stages of the parasites are minute and easily confused with droplets of fat or albuminoid corpuscles which often escape from the fat body of the slightly compressed larva.

After selecting living larvae which I suspected to contain the early stages of the parasites, I proceeded to make smears of their bodies, which after fixation and staining, revealed, with very few exceptions, the early stages of this parasite.

In this way I have collected a fairly rich material showing the various stages of the parasite. This material was studied in the form of smears as well as in sections of the larva.

As to technique, I may state that all classical methods of Protozoology were used. I have obtained the best results from the smears fixed in Schaudinn's fluid (with the addition of 1 per cent. acetic acid) and stained in iron-haematoxylin and from sections of the larvae fixed in Carnoy's fluid and stained also in iron-haematoxylin or in haemalum.

II. THE LIFE-HISTORY OF *HELICOSPORIDIUM PARASITICUM*.

1. *Localisation of the parasite.*

All the stages of the parasite are usually found free in the body cavity of the host. In several cases, however, especially when the infection was only recent, the parasites were found either in the fat body or in nerve ganglia. When they attack the fat body, the latter is rapidly destroyed and the parasites, attached to the fat droplets, escape into the body cavity. On the contrary when the nerve ganglia are infected, the infection remains for a long time localised; all the stages are then present simultaneously in the ganglia which become swollen and reduced to the neurilemma. It is interesting to remark that several successive nerve ganglia of the ventral chain may be infected, but the parasites are never found in the nerve commissures.

2. *Early stages and schizogony.*

The youngest stage of the parasite found in the tissue or in the body cavity of the host, is represented by small round corpuscles of 2 or 3 μ in diameter (Pl. IV, figs. 1, 2, 3); they are sometimes oval in shape, being then 3 μ long and 1.5 μ wide. The protoplasm of these corpuscles is homogeneous, being devoid of granulations and vacuoles. The nucleus, in the form of a spherical chromatic granule 0.5 μ in diameter is surrounded by a clear zone of protoplasm of 0.75 μ in diameter. This clear zone may be the real nucleus, while the chromatic granule is the nucleolus—this, however, could not be proved, as it was im-

possible to detect any nuclear membrane surrounding the clear portion of the protoplasm.

In this stage the parasite grows a little, and then divides into two (Pl. IV, figs. 3, 4, 5); the smallness of the parasite makes it very difficult, if not impossible, to follow in detail the mode of division. In some cases it appeared to me to be an ordinary amitotic division; in other cases on the contrary I could see a fairly clear mitosis. The two cells resulting from the division are of almost equal size and shape (Pl. IV, figs. 6, 7, 8 and 9); they are now more elongated and their protoplasm becomes more basophile. They grow a little, undergo a second division (Pl. IV, figs. 10, 11 and 12) and give rise to a small schizont (or morula) composed of four cells disposed in a tetrahedral manner (Pl. IV, figs. 13 and 14). In a few cases only I have observed all four cells symmetrically arranged quadrantly in one plane (Fig. 15). The schizont composed of four merozoites is usually slightly oval and measures 4μ by 3μ . These schizonts continue their development in two different ways: (1) either by breaking up into four merozoites (Pl. IV, figs. 16 and 17) which being set free, divide in their turn, or (2) by undergoing a third division (Fig. 18) and giving rise to schizonts composed of eight cells or merozoites (Pl. IV, figs. 19, 20). These schizonts, 4μ in diameter, are very basophile, so that it is often difficult to differentiate their nuclei. They undergo no further division, but break up into eight merozoites (Pl. IV, figs. 21 and 22), measuring 1.7μ – 2μ by 1μ which probably divide again in their turn.

I have not yet observed schizonts composed of more than eight cells. This multiplication, which forms an endogenous or schizogonic part of the life-cycle of *Helicosporidium* is very active and always results in the formation of an enormous number of unicellular corpuscles scattered throughout the body cavity of the host or invading its various tissues.

3. *Formation and structure of the spores.*

After a period of very active schizogonic multiplication the parasite passes into the second phase of its life-cycle, namely the formation of spores. The merozoite resulting from the schizogony increases slightly in size, becomes very basophile, and after two successive divisions (Figs. 23, 24 and 25) forms a morula of four cells tetrahedrally disposed. Of these cells, one grows more rapidly than the others and the whole morula completely loses its regular shape (Figs. 26, 27 and 28).

We now arrive at a very short phase in the life-cycle of the parasite, in which the latter undergoes some changes, the nature of which I was unable to follow clearly. However, by a few fragmentary observations and especially by the subsequent development, I think I have succeeded in reconstructing this missing stage, which I shall consider for the present as being hypothetical. Of the four cells which form the morula, one, the fourth, which lies now separately on one side and is much larger than the three other cells, finally

succeeds in surrounding them in the form of a ring. The three surrounded cells change their shape, becoming flattened, in the form of three superposed or parallel discs which occupy the centre of the ring formed by the fourth cell (Figs. 29 and 30). This cell then secretes an external membrane which envelops the whole group of four cells, thus forming a spore.

We now arrive at a stage in which the spore is most frequently encountered and which is easily recognised. It is the barrel-shaped spore surrounded by a very fine and transparent membrane or sporocyst. Its largest diameter is between 5 and 6 μ . When examined end-wise, the spore shows a central circular mass, strongly basophile, and surrounded by a highly refractive ring which fills the space between the central mass and the walls of the sporocyst (Pl. IV, fig. 31).

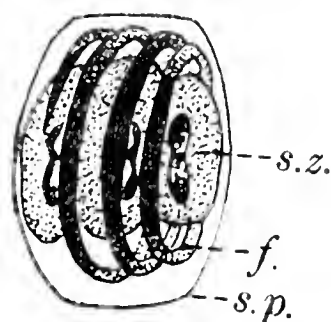
Examined from the side, the spore shows that the deeply-stained central mass is composed of three superposed discs, parallel to each other and to the flattened surfaces of the barrel-shaped spore (Pl. IV, fig. 32). These central discs are surrounded by a refractive ring which we now see from the side only.

Only by careful differentiation can the vesicular nuclei be detected in the central discoidal cells. These spores now undergo a further transformation: examined *in vivo* they show a spiral refringent band surrounding the three central cells and lining the sporocyst. The more detailed structure of this stage can be seen only in fixed and stained smears or in sections of the infested larva.

The protoplasm of the three central cells now loses more and more its basophile property, while in the refringent ring which surrounds these cells a chromatic substance appears which assumes the form of a spiral band with 3-4 turns surrounding the central cells of the spore (Text-fig. 1 and Pl. IV, figs. 33, 34, 35, 36 and 37). Viewed from the polar ends of the spore, the chromatic spiral band appears as a series of superposed chromatic rings surrounding the central cells (Pl. IV, figs. 38-42). During differentiation after staining with iron-haematoxylin, the chromatic spiral still retains a very dark colour, after all the rest of the spore is completely decolorised.

At this stage the nuclei of the central cells are distinct; the nuclei are variable in shape but are usually discoidal, their chromatin forming a peripheral ring which is connected with a central body or karyosome of an irregular form.

The parasite now invades the whole body of its host to such an extent that the latter dies, and as we have seen, the host's tissues are destroyed and replaced by a solid mass composed solely of these spores. For a long time I supposed that these spores represented the final developmental stage of the parasite, namely a resistant form, which, being set free from the dead host, were swallowed by other larvae of *Dasyhelea* which thus became infected.



Text-fig. 1. Completely formed spore showing the three central cells or sporozoites—s.z.; a peripheral spiral filament—f.; and the external membrane or sporocyst—s.p..

However, a further study of the parasite showed this supposition to be wrong, for the spores above described were found to undergo further development in the dead body of their host.

4. *Development of the spores subsequent to the death of the host.*

The decomposing sap collected from the wounds of trees often contains dead *Dasyhelea* larvae which, on microscopic examination are found to be completely filled with elongated filaments 60–65 μ long, with pointed extremities (Pl. V, figs. 8 and 9). As these filaments show a close resemblance to the acicular spores of yeasts of the genus *Monosporella*, one species of which I have described as being parasitic in the larva of *Dasyhelea*, I was at first under the impression that the elongated filaments were the spores of a similar yeast.

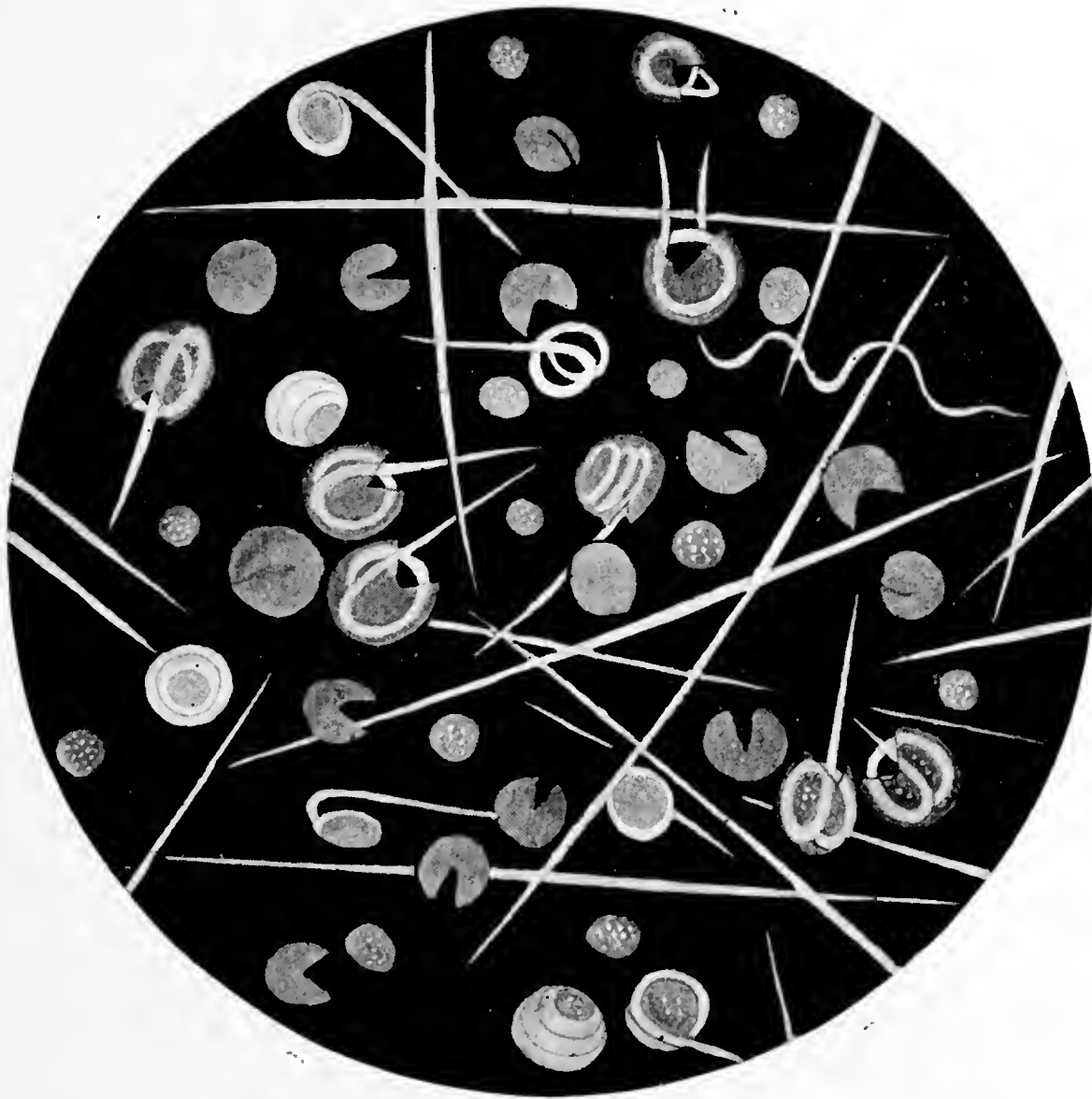
Subsequent observations showed me that such was not the case. These filaments have nothing to do with yeast, but actually belong to a later phase of the development of the spores of *Helicosporidium*. I have been successful in tracing the consecutive steps in the formation of these free filamentous structures from the barrel-shaped spores. If we isolate a parasitised *Dasyhelea* larva in a drop of ordinary tap water or in a small quantity of decomposed sap and leave it to itself it will soon die, being killed by the parasite. On allowing the body of this larva, filled with the barrel-shaped spores, to dry very slowly no noticeable change will occur in the spores; but if the dried body is moistened again, it swells up and a large number of filaments similar to those we have previously mentioned will appear among the barrel-shaped spores. The formation of these filaments does not necessarily result from a previous drying; it may occur also in the parasitised larva continuously submerged in water, but in this case the process takes place much more slowly. The successive drying and moistening of the spores appears, however, to hasten the formation of the filaments, and doubtless plays a very important part in nature, because the wound of the tree is necessarily exposed to alternating conditions of drought and moisture.

All the stages in the formation of these filaments are easily found by opening the body of the parasitised larva in a drop of water a few days after the insect's death (Text-fig. 2). The process whereby the barrel-shaped spore gives rise to filaments can only be followed, however, by examining smears prepared from the dead infected larvae, fixed with Schaudinn's solution and stained with iron-haematoxylin.

Such smears show very clearly that the filaments are the unrolled internal spirals liberated from the spores. The latter, under the pressure of the unrolling spirals, rupture at one end, and from the opening in the ruptured sporocyst a portion of the spiral protrudes (Pl. IV, figs. 42–47). The protrusion usually begins at one end of the spiral, which progressively unrolls and liberates itself from the sporocyst. At various stages of this unrolling the three central round cells are mechanically expelled from the spore, leaving the sporocyst completely empty.

In somewhat rare cases the protrusion of the spiral begins either with a loop (Pl. IV, fig. 44) formed by its central portion or with both extremities simultaneously (Fig. 46).

The same smears also contain many other spores devoid of a sporocyst (Text-fig. 3). The spiral filament of these spores is of a very irregular shape, the rings which form it being often loosened or the unrolled portion secondarily twisted. The irregular form of these filaments is undoubtedly due to mere mechanical pressure produced during the preparation of the smears.



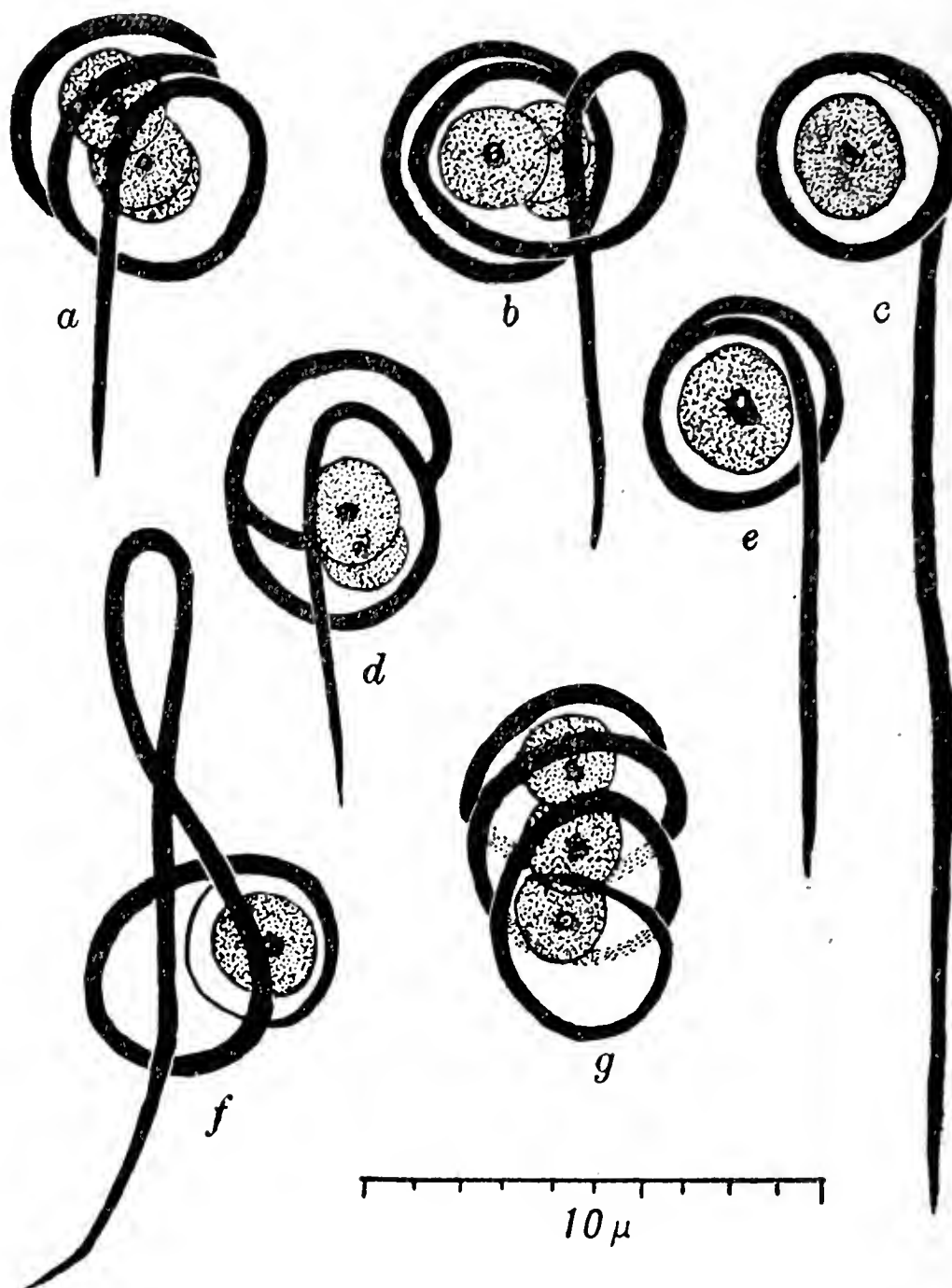
Text-fig. 2. A drop of fluid taken from the dead body of a *Dasyhelea* larva infected with *Helicosporidium*. The drop diluted with normal salt solution, shows different stages of the opening of the spores and unrolling of the spiral filaments.

The three liberated central cells, which are 2μ in diameter, remain much as they were when inside the sporocyst, with the difference that the refractive granules in their protoplasm are now more distinctly visible.

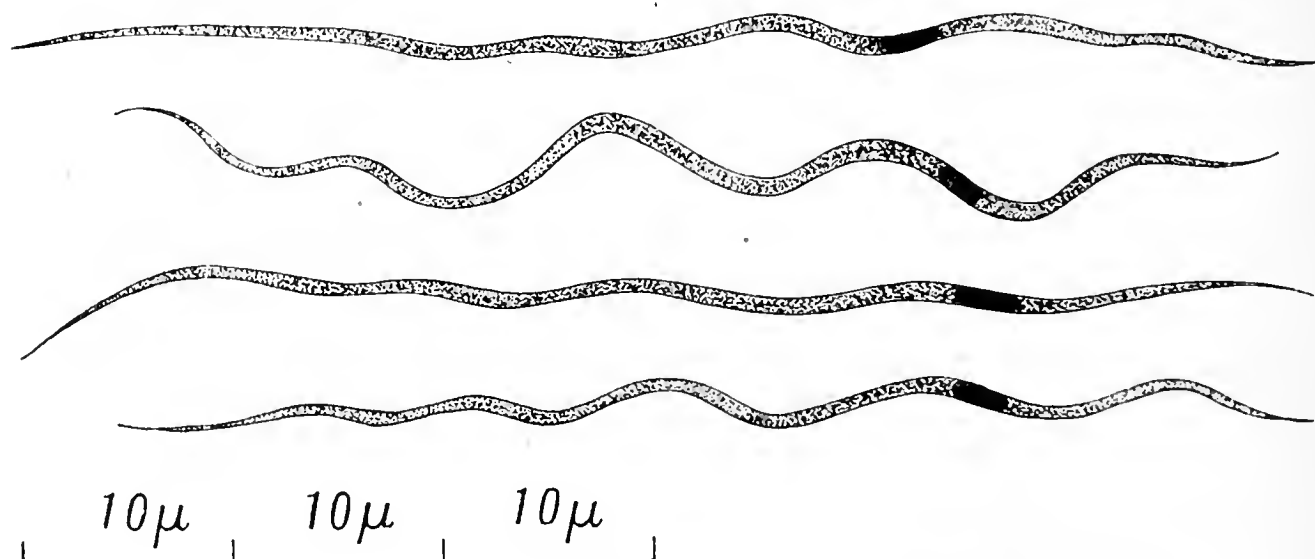
The structure and size of the filament are fairly uniform. Examined *in vivo* they are straight, needle-shaped (Text-fig. 2 and Pl. V, figs. 8 and 9), $60\text{--}65\mu$ long and 1μ wide. They are very refractive, pointed at both ends, but the two extremities are not equally attenuated.

In fixed and stained smears, these filaments are more or less sinuous and much narrower, not exceeding 0.65μ in their widest portion (Text-fig. 4, Pl. V, fig. 7). This is undoubtedly due to the fact that the central axial

part of the filament takes the stain, while the peripheral refractive non-staining sheath either contracts or becomes invisible in refractive mounting



Text-fig. 3. Different forms of unrolling spiral filaments without sporocyst; such forms are often found in fixed and stained smears.



Text-fig. 4. Unrolled filaments in fixed and stained smears showing their nuclei.

media. In iron-haematoxylin the filaments stain very slowly, but once stained, they are very retentive of the dark colour and are decolorised with difficulty.

After differentiation in iron alum, the darkly stained axial portion of the filament presents a granular structure, and if the differentiation is more prolonged, until the filament becomes of a grey colour, a darkly stained chromatic body, of definite size and structure, makes its appearance (Text-fig. 4). This body, which is undoubtedly a nucleus, is $2-3\mu$ long and 0.6μ wide, always lies in the wider portion of the filament, $15-18\mu$ from its extremity. By prolonged differentiation this nucleus can also be detected in filaments of the spiral form while still enclosed in the sporocyst (Pl. IV, fig. 41). We now arrive at the final stage of the life-cycle of the parasite: the distended cuticle of the dead larva being filled with a felt-like mass of entangled filaments mixed with small cells, the central cells of the spores and the empty sporocysts (Pl. V, figs. 8 and 9).

At this stage the macerated cuticle of the larva breaks away and its entire contents escape into the surrounding medium, the decomposed sap of the tree. Smear preparations of this sap often reveal the above-mentioned filaments, while the central cells of the spores cannot be recognised in the crowd of various micro-organisms, yeasts, moulds, rhizopods, ciliates, etc., which usually inhabit the fluid.

5. *Supposed mode of infection of the host.*

A question arises now: which is the infective form of *Helicosporidium*? Is it the filament or the central cells of the spore? The cellular structure of the filament, its great resistance to external influences, and its resemblance to the infective stage of *Monosporella* suggest that it may be the infective stage. If this is the case, the central cells of the spore could only be considered as residual bodies.

On the other hand, the great number of the central cells (three times more numerous than the filaments) and their resemblance to the first stages of *Helicosporidium*, as seen in the body-cavity of the host, make it almost certain that they represent the real sporozoites or infective forms of the parasite. In this case the spiral filament may be regarded as a cell differentiated for the purpose of dehiscence of the spore and can be compared in respect to its function with the elaters of Mycetozoa, with the difference that, while in the latter they are of complicated structure and extrasporal, in *Helicosporidium* they are unicellular and intrasporal.

The sporozoites, after being swallowed by a healthy larva, penetrate probably through the wall of the alimentary canal into the body cavity of the larva, where they begin their endogenic multiplication or schizogonic cycle.

6. *Frequency of infection of Dasyhelea larvae.*

Several generations of *Dasyhelea obscura* occur in the course of a year, and the larvae of all the generations are equally subject to infection. It is, however, impossible to estimate the true rate of infection as this varies greatly and depends upon the condition of the wound of the tree at the time

when the material is collected. In rainy weather the larvae leave the flooded parts of the tree's wounds and penetrate into the fissures of the tree; in the meantime, the wound is thoroughly washed by the rain and is freed from the collected sap which usually contains dead and dying larvae infected with *Helicosporidium*. When the normal conditions are restored and the wound is once more covered with freshly exuded sap, the larvae crawl again from their hiding places and invade the wound. If the sap is collected at this time, very few infected larvae will be found. On the contrary, in damp weather with the absence of much rain, when the old sap remains in the wound for a prolonged period, the number of diseased larvae increases. Finally, the sap collected from the wounds and kept in jars in the laboratory gives a still higher proportion of diseased larvae, as in this case the non-infected larvae rapidly become infected from contact with their diseased companions.

7. Stages of the host susceptible to infection.

In all its larval stages *Dasyhelea obscura* is susceptible to infection with *Helicosporidium*. This is undoubtedly due to the feeding habits of the larvae remaining uniform throughout its life. Very small larvae, hardly 1.5 mm. long, were often found with the body cavity filled with spores of the parasite, the spores showing already completely formed spirals and the three sporozoites. In a single instance a full-grown larva, almost ready to pupate, showed only the schizogonic cycle of the parasite, a condition which indicates a recent infection. Between these two extremes all the intermediate phases are met with. Only a few pupae of *Dasyhelea* were found infected and this infection is almost certainly derived from the larval stage: a full-grown larva became infected just before pupating, when all its imaginal discs were already completely formed and the pupation took place before the parasite had time to make a destructive invasion. Such infected pupae are eventually killed by the parasite. In no case have I observed the parasite in the adult insect.

III. OTHER HOSTS OF *HELICOSPORIDIUM PARASITICUM*.

1. *Mycetobia pallipes* Meig. (Diptera, Rhyphidae).

The larvae of *Dasyhelea obscura* are usually found associated with a number of other Dipterous larvae living in the same medium. According to their feeding habits, these larvae can be separated into two groups:

(1) Saprophagous larvae which like *Dasyhelea* feed upon the decomposed sap e.g. *Rhyphus fenestralis* Scop., *Mycetobia pallipes* Meig., *Aulacogaster rufitarsis* Meig. and the larvae of *Eristalids*.

(2) Carnivorous larvae such as *Systemus adpropinquans* Loew, and *Phaonia cincta* Zett.

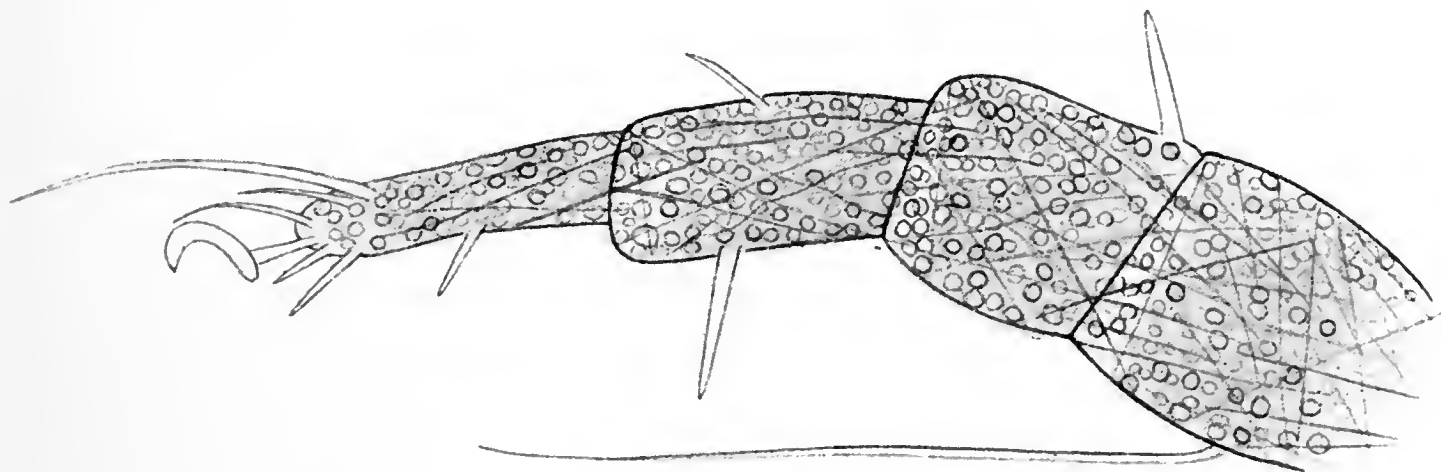
I have frequently examined large numbers of larvae of all these different species for various parasites and only once have I found the spores of

Helicosporidium in a specimen of *Mycetobia pallipes*, invading the peripheral portion of its fat-body.

2. *Hericia hericia* (Robin) Kramer (1899) (Acarina, Tyroglyphidae).

In addition to the above-mentioned Dipterous larvae, the exudate which fills the wounds of the elm tree contains also a very interesting mite belonging to the family Tyroglyphidae. This mite, *Hericia hericia* (Robin) Kramer, 1899, is undoubtedly the most frequent inhabitant of the exudate. It was discovered and very well described by Robin (1868) who found it in the exudate of elm trees in France.

The mite has been since found in England by Michael (1903, Vol. II. "Tyroglyphidae," pp. 31-38, Pls. XXIII-XXIV) who gives in his monograph of British Tyroglyphidae a complete description of all its stages. Concerning its habitat he writes: "This species usually lives in a semi-aquatic condition, wading in the sap which exudes from splits in the bark of elm trees, or under loose bark of these trees, and in the brown saccharine matter which collects there. In such situation it is often present in great numbers; it is also found,



Text-fig. 5. A leg of *Hericia hericia* heavily infested with *Helicosporidium* showing the spores and filaments

but less frequently, in similar situations on the oak. I have not found it on other trees, but it is quite possible that it may exist on them."

I myself found this mite abundantly, often covering the entire surface of the wound.

On many occasions I have carefully examined large numbers of this mite without finding a single parasitised specimen, but recently (October, 1920), whilst collecting the *Dasyhelea* larvae, I noticed a portion of the body of *Hericia* with two legs attached to it, the whole filled with filaments and a few complete spores of *Helicosporidium*. I believed at first that I was dealing with an empty skin of a dead *Hericia* which was invaded by a small *Dasyhelea* larva parasitised and killed by *Helicosporidium*, but on careful examination of other mites, I soon discovered eight entire specimens, three of which were alive, all showing *Helicosporidium* in different phases of its life-cycle.

Text-fig. 5 shows a leg of a very heavily infested specimen of *Hericia*, the whole body of which is invaded with the spores and a large number of filaments.

All this shows clearly that *Helicosporidium parasiticum*, although a common parasite of *Dasyhelea obscura*, is by no means specific to this host, but occurs in other Dipterous larvae like *Mycetobia pallipes* and what is still more remarkable, in at least one other Arthropod, the Tyroglyphid mite *Hericia hericia* (Robin) Kramer, 1899.

IV. SYSTEMATIC POSITION OF THE GENUS *HELICOSPORIDIUM*.

Now that we know the structure and the life-history of *Helicosporidium*, a question arises as to the systematic position of this genus. It seems to me very difficult to answer this question and all I can do at present is to discuss the relations between the new parasite and various forms of Protists.

1. *Helicosporidium* and *Cnidosporidia*.

One is tempted first of all to compare the genus *Helicosporidium* with the Sporozoa, especially those which have multicellular spores, as for instance Cnidosporidae, comprising the three Orders: Myxosporidia, Actinomyxidia and Microsporidia.

The trophozoite stage of *Helicosporidium* as well as its schizogonic cycle recalls in many respects those of the Microsporidia Monosporogenea Pérez (*e.g.* *Nosema bombycis*); on the other hand, the trophic stage, in the form of a small round cell, as well as the schizogonic cycle, cannot be used for establishing the affinities between the various groups of Protists as similar modes of multiplication can be found in widely separated orders.

On the other hand the development and the structure of the spores provide a series of much more important characters which have been used already with success in the classification of the Protozoa. A character which is common to all Cnidosporidia and *Helicosporidium* is the complicated multicellular structure of the spores, the latter in both cases being composed of heterogeneous elements. It remains to be seen, however, in how far the spore cells of *Helicosporidium* can be compared with those of the Cnidosporidia.

It may be assumed, for instance, that the three central cells or sporozoites of *Helicosporidium* correspond to the germ cells, sporoplasm or sporozoites of the Cnidosporidia, while the polar capsule of the latter corresponds to the spiral filament of our parasite.

It must be admitted, however, that this assumption, which at first sight appears to be reasonable, is based on very superficial points of resemblance, and it needs a critical examination. The germ cells or sporoplasm of the Cnidosporidia differ from those of *Helicosporidium* in that they are usually reduced to a single binucleated cell, instead of three uninucleated cells as in *Helicosporidium*.

Among the Cnidosporidia the uninucleated sporozoites are known in a few species of Actinomyxidia, but they are very numerous in each spore.

As to the filament of *Helicosporidium*, although it is formed from one

differentiated cell as is the polar capsule of Cnidosporidia, it differs from the latter in several essential characters.

The following table shows the points of difference between these two structures:

Spiral filament of <i>Helicosporidium</i>	Polar-capsule filament of Cnidosporidia
(1) Filament not enclosed in a polar capsule but lies free beneath the wall of spore.	(1) Filament enclosed in a capsule of which it forms a part.
(2) Filament always unrolls in the dead body of its host.	(2) Filament does not unroll until spores reach intestine of a second host.
(3) Filament unrolls slowly.	(3) Filament is shot out.
(4) Filament pointed at both ends and wide and ribbon-like in the middle.	(4) Filament pointed at one end only and very fine.
(5) Axial portion of filament is very chromatic, nucleus well formed in anterior third of filament.	(5) No chromatic axial portion, degenerated nucleus upon wall of terminal capsule.
(6) Filament robust, very resistant in all media.	(6) Filament fine and very fragile.

The foregoing shows clearly that the spiral filament of *Helicosporidium* is of a nature totally different from that of the polar capsule of the Cnidosporidia.

Other distinctive characters of *Helicosporidium* are: (1) the wall of the spore does not seem to be formed by a specialised cell, at any rate no trace of such a cell could be detected in the wall of the completely formed sporocyst; (2) the spore of *Helicosporidium* does not show a binary or ternary symmetry and pansporocysts are non-existent.

All this demonstrates clearly enough that there is no real affinity between the genus *Helicosporidium* and the Cnidosporidia.

2. *Helicosporidium* and *Haplosporidia*.

We may now compare the genus *Helicosporidium* with the Order Haplosporidia of Caullery and Mesnil (1905). Although this Order, conceived in its widest sense, is heterogeneous, all the forms which it comprises differ greatly from *Helicosporidium*. In their life-cycle they have a plasmodium stage and a cyst which surrounds the spores; these characters never appear in the life-history of *Helicosporidium*. The spores of the Haplosporidia are unicellular with one or two envelopes while the spores of *Helicosporidium* are composed of four cells of different structure. These differences are sufficient to show that there is no affinity between *Helicosporidium* and the Haplosporidia.

3. *Helicosporidium* and *Serumsporidia*.

It remains finally to be seen whether or not there are some relations between *Helicosporidium* and a few Protists temporarily placed in the Sporozoa but whose systematic position is still *subjudice*. The only group among these Sporozoa which may interest us is the group of Serumsporidia of Pfeiffer.

Under the generic name of *Serumsporidium*, Pfeiffer (1895) has described a certain number of parasites which he discovered infesting the body cavity

of several species of Crustacea, belonging to the genera *Cypris*, *Daphnia* and *Gammarus*.

The descriptions and figures of this author are, however, very superficial and incomplete, and it is hardly possible to get a general idea of the structure of the parasites which he has described.

Neither his descriptions nor his figures of some eight distinct species of this genus give the remotest indication as to the character which these species have in common. From his description one can only say that the few characters which are common to all his eight species are of no systematic value, namely (1) that these species live as parasites in the body cavity of Crustacea, (2) that they produce very numerous spores, (3) that their structure and life-cycle are equally obscure.

From the systematic point of view the term *Serumsporidium* has no more value than the term "blood parasites" applied to protozoal parasites of mammals.

It is almost certain that his "*Serumsporidium II* (Mülleri), nov. sp.," "*Serumsporidium gammari*" and especially "*Serumsporidium cypridis IV*, n. sp." are the spores of a gregarine liberated from the ripe cysts, which, being fragile, have become ruptured inside the body of the host.

From all his descriptions and figures those relating to his species "*Serumsporidium* (*Cytamoeba*? Labbé) *cypridis I*, nov. sp." (p. 12), are of especial interest to us, as three of the figures illustrating this species bear some resemblance to *Helicosporidium*. His figure 2, B. 7 (p. 12), for instance, recalls the morula of *Helicosporidium* composed of four cells, one of which is concealed behind the other three, while the Figs. 2, B. 8 and B. 9, resembles somewhat the spores of *Helicosporidium* which shows the three nuclei of the central cells only. It is, however, hardly possible to base upon these superficial resemblances any relationship between *Serumsporidium* and *Helicosporidium*.

4. *Helicosporidium* and *Mycetozoa*.

Helicosporidium has no affinities with the *Mycetozoa*, several species of which have been already found parasitic in insects. It differs from the *Mycetozoa* in the absence of the plasmodium and flagellate stages and by the complicated structures of the spores, which, on the contrary, are simple in *Mycetozoa*.

V. CONCLUSIONS.

The foregoing evidence shows clearly that the genus *Helicosporidium* differs markedly from all the actually known Protists, and that it forms a new type which may be temporarily included in the group of the Sporozoa. It is possible that the discovery of other new forms of Protists, parasitic in insects, will throw more light upon the systematic position of *Helicosporidium* or will lead to the finding of a connecting link between this new genus and the already well-known forms.

The genus *Helicosporidium* may be characterised as follows;

Helicosporidium n. g.

Diagnosis. Parasitic protist; trophic stage in the form of a small round cell $2-3\mu$ in diameter, with small spherical nucleus. Schizogonic multiplication very active. Schizonts forming a small morula of 4μ in diameter, composed of four or eight merozoites, which become free.

Spore ($5-6\mu$ in diameter) is composed of four cells surrounded by a thin wall or sporocyst. Of the four cells, three, which form the real sporozoites, are discoidal, occupying the centre of the spore. The fourth cell forms a peripheral spiral filament which surrounds the central cells.

The spores open inside the dead body of their host, by the unrolling of the spiral filament, and the sporozoites are thus liberated.

The spiral filament when unrolled is $60-65\mu$ long, pointed at both ends and 1μ thick at its widest portion; its nucleus $2-3\mu$ long, lies 15μ from one end of the filament. This filament is of a very resistant nature. One species known.

Helicosporidium parasiticum n. sp.

Diagnosis. The same as that of the genus.

Habitat. Body cavity, fat body and nervous system of the larva of *Dasyhelea obscura* Winnertz (Diptera, Nematocera, Ceratopogonidae). Occurs also in *Mycetobia pallipes* Meig. (Diptera, Nematocera, Rhyphidae) and in *Hericia hericia* (Robin), Kramer, 1899 (Acarina, Tyroglyphidae). The hosts inhabit wounds of elm and horse-chestnut trees, Cambridge, England.

Acknowledgements. I am much indebted to Mr L. E. Robinson, who is engaged in research in the Quick Laboratory, for the very friendly help that he has given me in the preparation of this paper for the press and especially for the great pains he took in the preparation of the accompanying photomicrographs (Pl. V).

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EXPLANATION OF PLATES IV—VI.

Helicosporidium parasiticum.

PLATE IV.

All the figures of this plate relate to the smears fixed in Schaudinn's solution with 1 per cent. of acetic acid added, and stained in iron-haematoxylin and eosin. Owing to the small size and complicated structure of the spores, high magnification was used for this study: Apochromatic imm. object. 2 mm.; N.A. 1.4 with Comp. oculars 8 and 12. For the sketches which were made with the camera lucida, comp. ocular 18 was used; the scale of magnification common to all the figures is shown in the plate beneath the figures.

Figs. 1, 2, 3. Trophic stages of parasite.

Figs. 4, 5. First division of the parasite and formation of two cells.

Figs. 6–9. Different forms of bicellular stage.

Figs. 10–12. 2nd division and formation of schizonts of four cells.

Figs. 13, 14. Schizonts composed of four cells, tetrahedrally disposed.

Fig. 15. Exceptional forms of four cellular schizonts with merozoites radially disposed.

Figs. 16, 17. Breaking up of four-celled schizonts into four merozoites.

Fig. 18. Nuclear division in schizonts of four cells.

Figs. 19, 20. Schizonts composed of eight cells.

Figs. 21, 22. Breaking up of eight-celled schizont into eight merozoites.

Figs. 23, 24, 25, 26. Three successive stages in formation of morula of four cells tending to formation of the spores.

Figs. 27, 28. Further transformation of these cells showing one cell growing more rapidly and surrounding the three other cells.

Fig. 29. Obscure stage showing a central mass surrounded by a ring.

Fig. 30. Hypothetical figure showing the three central discoidal cells surrounded by the fourth cell.

Fig. 31. A young spore, end view, showing a central darkly stained body composed of three superposed discoidal cells, surrounded by a very refractive ring, and by a thin wall or sporocyst.

Fig. 32. Side view of a similar spore showing clearly the three central cells.

Fig. 33. Further development of the spore (as shown in Fig. 32), showing the spiral filament formed in the refractive ring surrounding the three central cells or sporozoites.

Figs. 34–37. Spores viewed side-wise, similar to that illustrated in Fig. 33, showing some variation in size (due to fixation) and in the appearance of nuclei of the central cells.

Figs. 38–40. End view of a similar spore, the figs. 39 and 40 do not show the sporocyst, this being due to contraction by the fixative.

Fig. 41. Spore stained and much decolorised, showing a nucleus in the spiral filament.

Fig. 42. Spore seen from one end in a smear where almost all the other spores are already open (derived from the dead larva).

Figs. 43, 44. Ruptured spores showing the protrusion of a portion of the filament.

Fig. 45. Protrusion of the filament through the opening of a ruptured sporocyst.

Fig. 46. A case of protrusion of both ends of the spiral filament.

Fig. 47. More advanced stage of protrusion of the filament and its unrollment.

PLATE V.

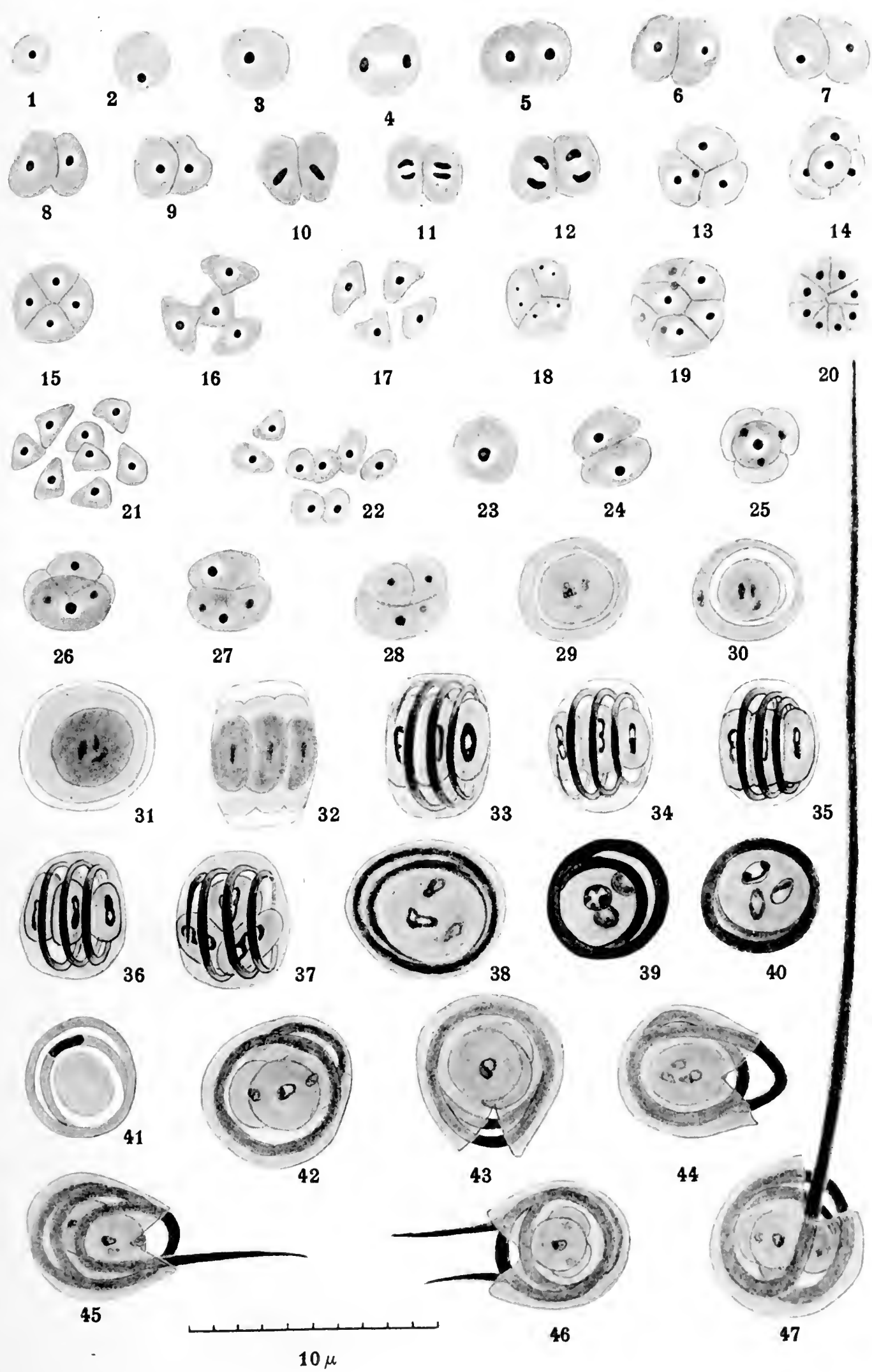
Photomicrographs taken by Mr L. E. Robinson.

Fig. 1. Living larva of *Dasyhelea obscura* heavily parasitised, pressed between the slide and a coverslip until the posterior end of its body was ruptured, projecting an enormous mass of spores. The latter can be seen still filling the body cavity of the larva and reaching into the head. $\times 75$.

Fig. 2. The upper portion of the same larva under a higher magnification. $\times 150$.

Fig. 3. Another larva, filled with spores of *Helicosporidium* and ruptured in its middle, showing the spores inside and outside the body. $\times 150$.

Fig. 4 $\times 800$. Fig. 5 $\times 300$. Fig. 6 $\times 250$. Fig. 7 $\times 500$. Fig. 8 $\times 100$. Fig. 9 $\times 400$.







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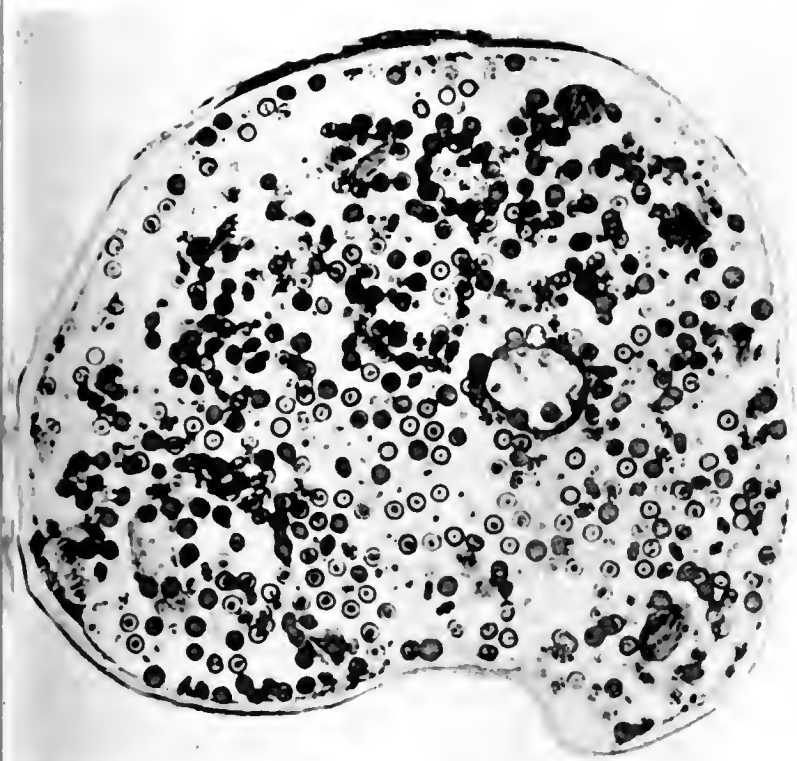
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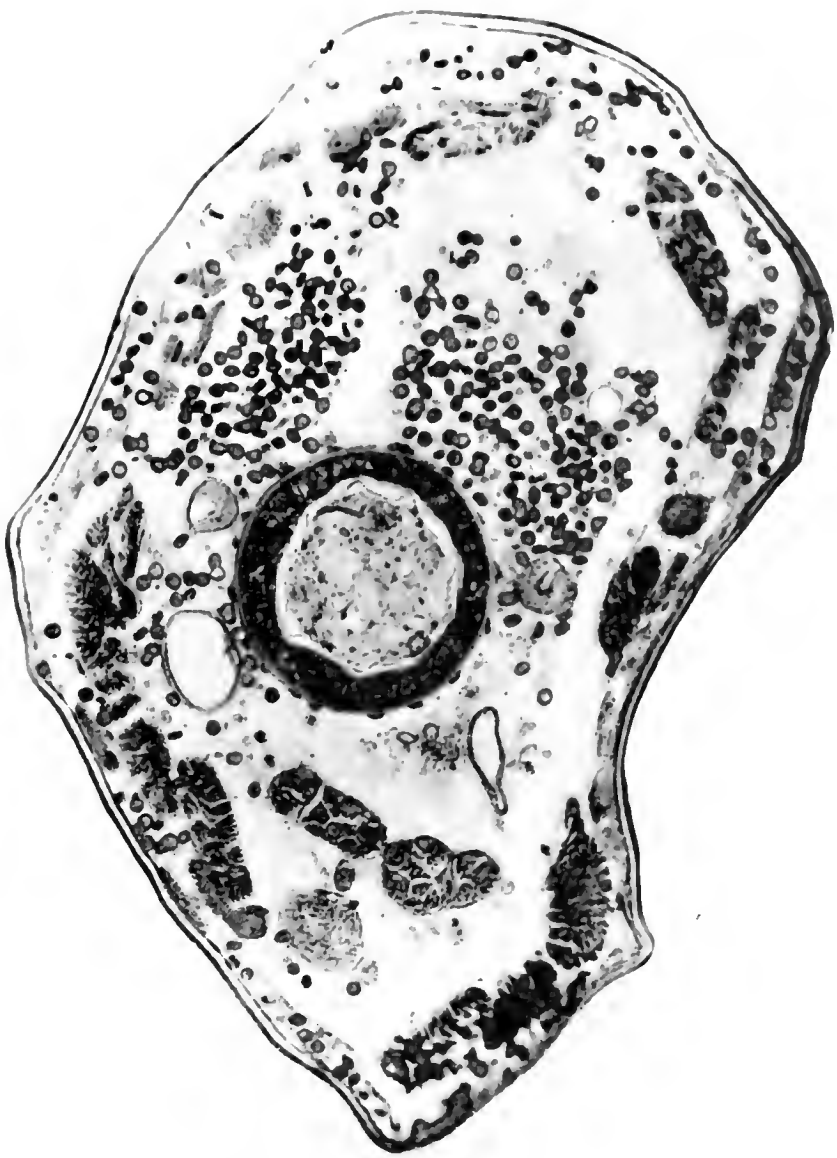
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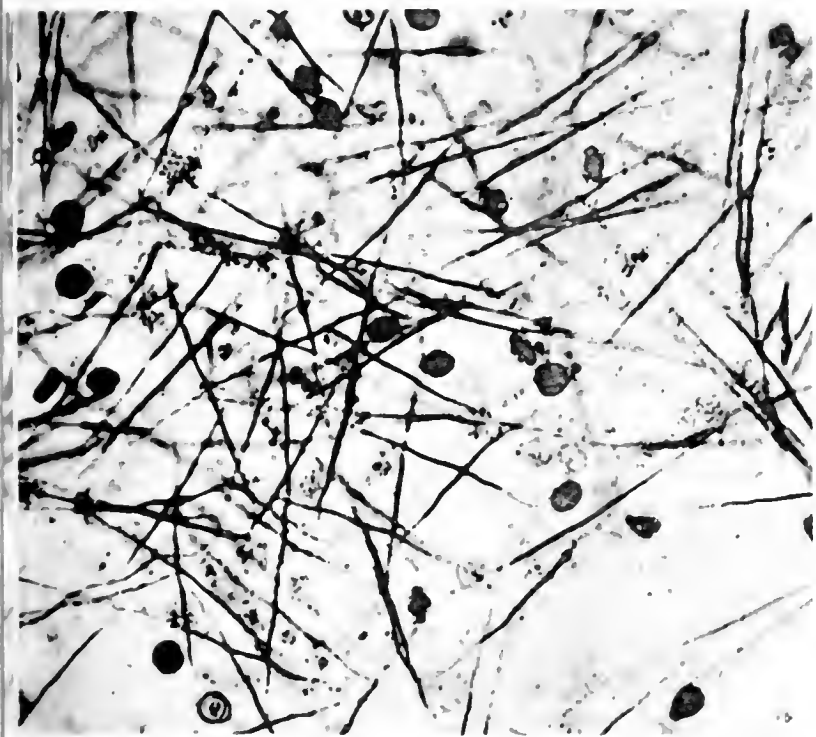
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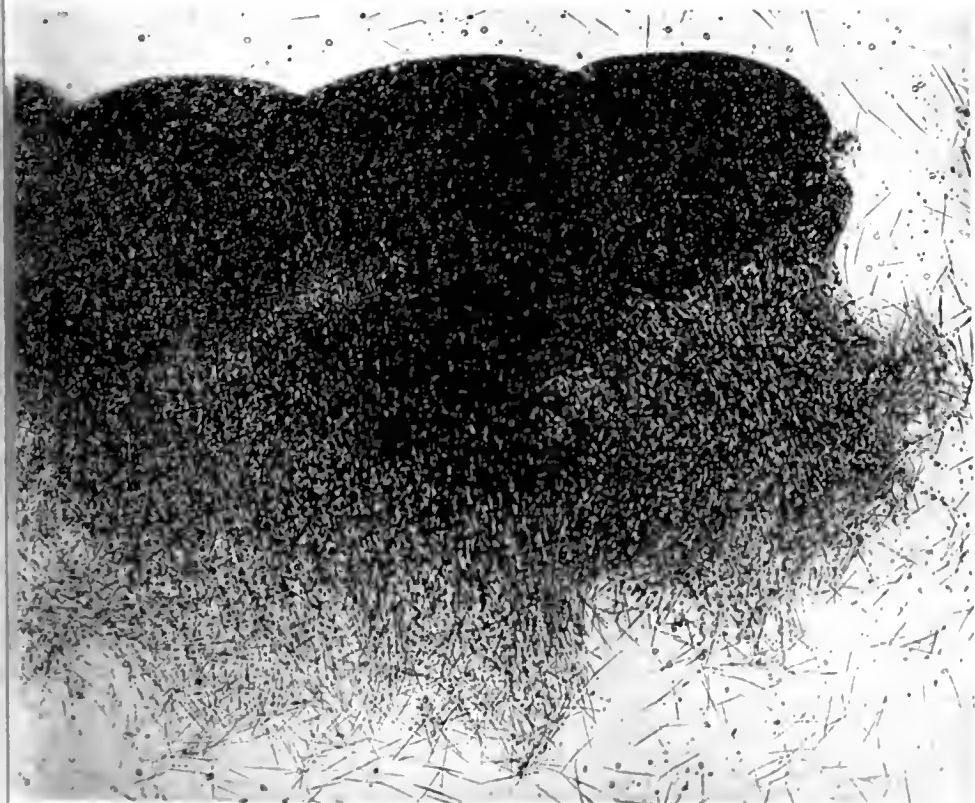
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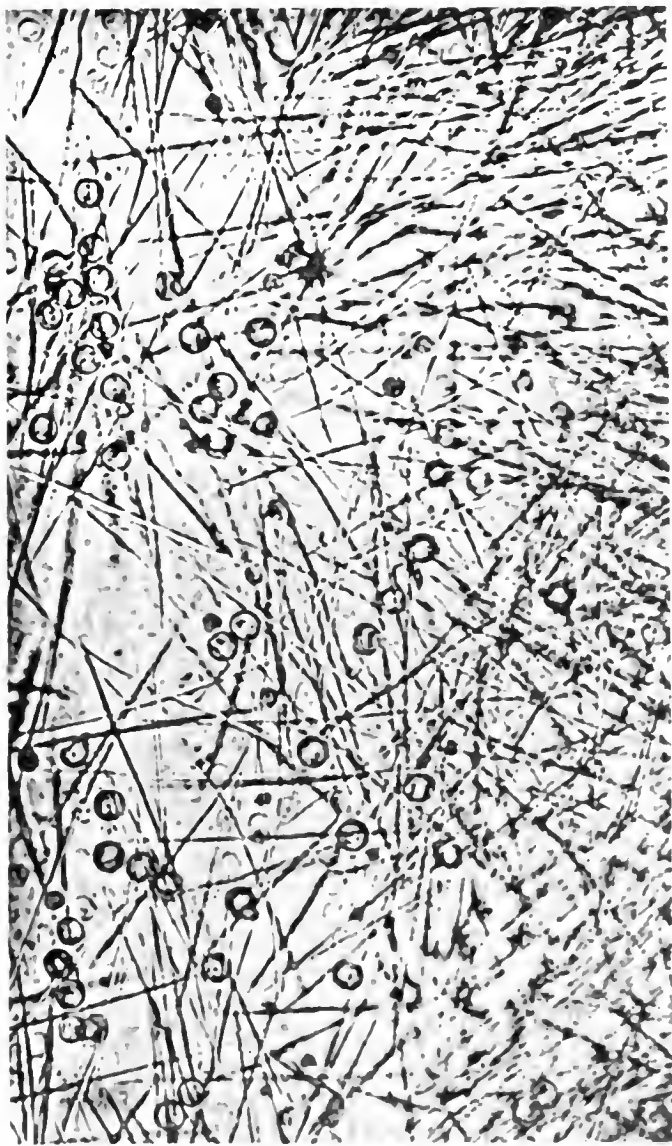
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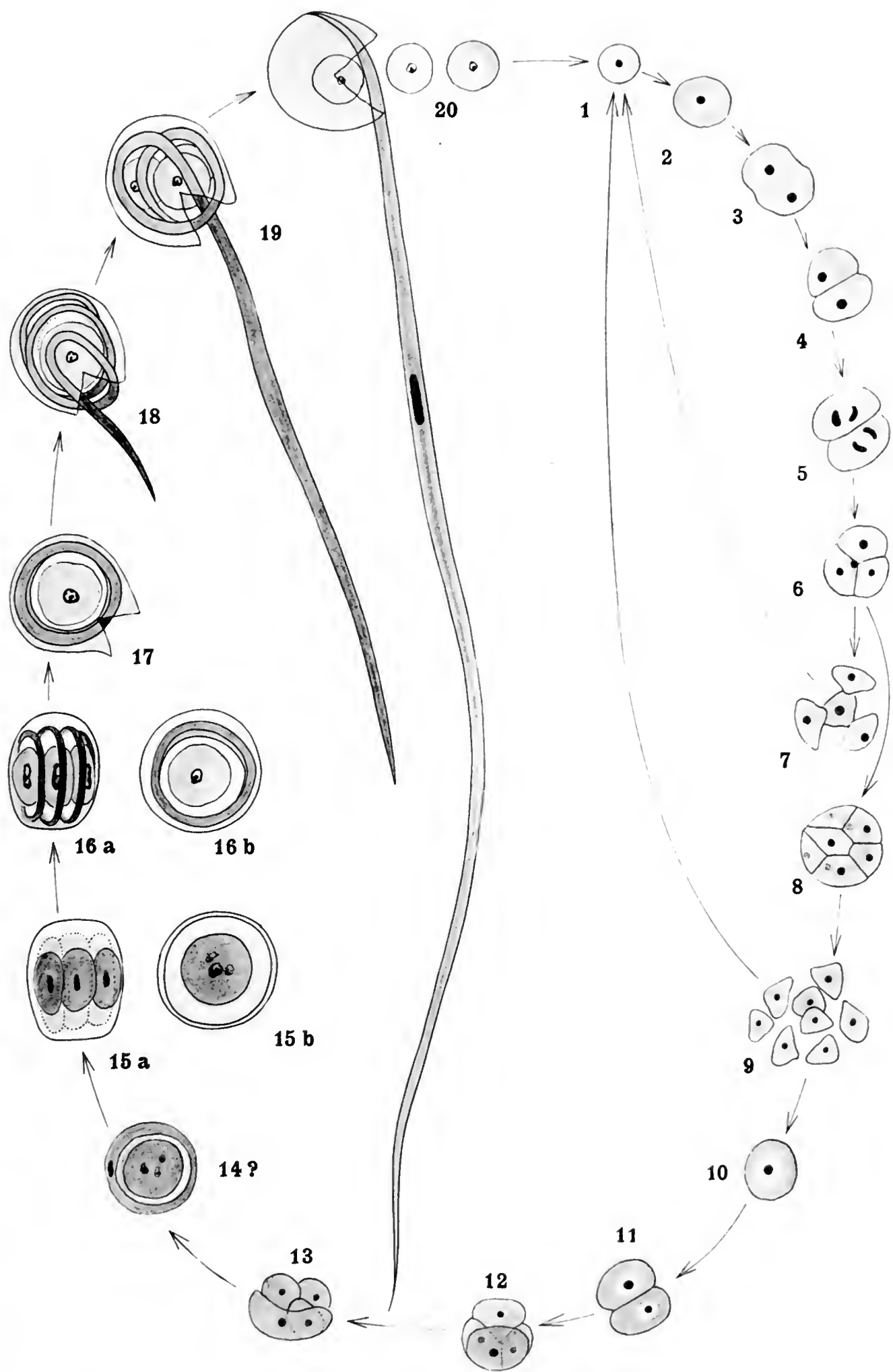
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- Fig. 4. Smear, fixed and stained, showing the spores with central cells and spirals.
 Fig. 5. Transverse section of *Dasyhelea* larva very heavily infected with *Helicosporidium*.
 Fig. 6. Transverse section of *Dasyhelea* larva not heavily infected with *Helicosporidium*.
 Fig. 7. Spores and unrolled filaments of *Helicosporidium* in fixed and stained smears.
 Fig. 8. Dead body of *Dasyhelea* larva infected with *Helicosporidium*, the spores of which are open and the spiral filaments free and unrolled. The body of the larva is ruptured by the pressure of a coverslip and shows an enormous number of escaping filaments.
 Fig. 9. A portion of the same mass of filaments under a higher magnification.

PLATE VI.

Life-cycle of *Helicosporidium parasiticum*.

- Figs. 1-9. Different phases of schizogonic multiplication, formation of four- and eight-celled schizonts.
 Figs. 10-13. Formation of four-celled morula which develops into the spores.
 Fig. 14. Hypothetical stage showing the three central discoidal cells surrounded by the fourth cell.
 Fig. 15. Young spore before the formation of spiral filament: (a) side view, (b) end view.
 Fig. 16. Mature spore: (a) side view, (b) end view.
 Figs. 17-20. Different stages of the opening of the sporocyst, unrolling of the spiral filament and liberation of sporozoites.

Stages 1-16 occur in the living larva of *Dasyhelea*, while the stages 17-20 are found only in the dead body of the host.

THE EXTERNAL ANATOMY OF THE *SARCOPTES*
OF THE HORSE¹.

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(*From the Quick Laboratory, Cambridge.*)

(With Plate VII and 22 Text-figures.)

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INTRODUCTION.

WARBURTON (1920) has recently published a survey of our present knowledge of the mites of the genus *Sarcoptes*; from his survey it is clear that in spite of all the work that has been done upon these mites, much of it by most painstaking anatomists, we are still without an accurate knowledge of the anatomy of any one species of *Sarcoptes*. This is, in part at any rate, due to the fact that some of the best work was done, by Robin and others, between 1860 and 1865, at a time when microscopes and technique were by no means as highly developed as they now are.

This paper is an attempt to carry forward an investigation, the main lines of which have been indicated by Warburton; to describe as accurately as possible the anatomy of one *Sarcoptes*, that of the horse, and to illustrate its structure. A detailed knowledge of the structure of one species of *Sarcoptes* is desirable in order that the validity of the numerous very similar species may be settled once and for all. In this paper I purposely avoid reference to systematic

¹ Work done with the aid of a grant from the Ministry of Agriculture and Fisheries.

matters and deal only with the *Sarcoptes* of the horse, terming it *S. scabiei* var. *equi* Gerlach, without prejudice to the question of its rank as variety or species. I shall be glad to receive at the Quick Laboratory, Cambridge, specimens of *Sarcoptes* or other mange mites from any animal; the specimens should be sent alive if possible. When sufficient material has accumulated I hope to carry out a comparative study of the *Sarcoptes* of various animals.

I shall not deal with the life-history of *S. scabiei* var. *equi*. A good biological study of the closely allied, if separable, *S. scabiei* var. *hominis* has recently been published by Munro (1919), and, since it bears on my anatomical studies of the various stages of *S. scabiei* var. *equi* described in this paper, an outline of the life-cycle will be useful. From the egg hatches the larva, and from the larva emerges the nymph; I do not think that there is any external difference to be found between male and female. It is believed that the nymph, on moulting, produces either an adult male or an immature female. It is currently held that the immature female is the stage in which copulation occurs, but I have produced evidence (p. 139) for believing that in *Sarcoptes* the act is performed only by the adult female. We believe then that in the male there are three stages after the egg, and in the female four, that is to say the larva, the nymph, the immature female and the adult female.

THE ADULT FEMALE.

The following measurements are taken from ten females measured. Length from tip of chelicera to end of body, 357–432 microns (average 388 microns); breadth 250–295 microns (average 271). The ratio of length to breadth varies between 100/79 and 100/66, the average of the ten being 100/70. It will be seen that there are considerable individual differences not only in absolute size, but also in the relation between length and breadth. These differences are in all probability due to the action of the great longitudinal and dorsi-ventral muscles, which must be capable of producing great alterations in the shape of the mite.

Method. All the specimens were measured alive, and then cleared in gum arabic in order that I might satisfy myself by the examination of the tocostome that I was dealing with adult females. It is essential that this method should be followed in measuring *Sarcoptes* because it is readily compressed by a cover-slip, and altered in size and shape by fixation. According to the earlier authors there are considerable differences in size and shape between the various species of *Sarcoptes*, but we do not know how many individuals they measured nor under what conditions, so that it is doubtful whether the measurements of one author are comparable with those of another. The dimensions of *S. scabiei* var. *equi*, as given by Canestrini and Kramer, are length 450–500 microns, breadth 310–370 microns. These measurements are considerably in excess of my own, and were possibly taken from mounted specimens. Gerlach gives length 440, breadth 300 microns for the female, figures much closer to my own.

The body. The general shape of the body may be seen from Fig. 1. Subject to considerable individual differences it is roughly oval, and widest slightly behind the middle. It is irregularly convex above, flatter beneath and of a

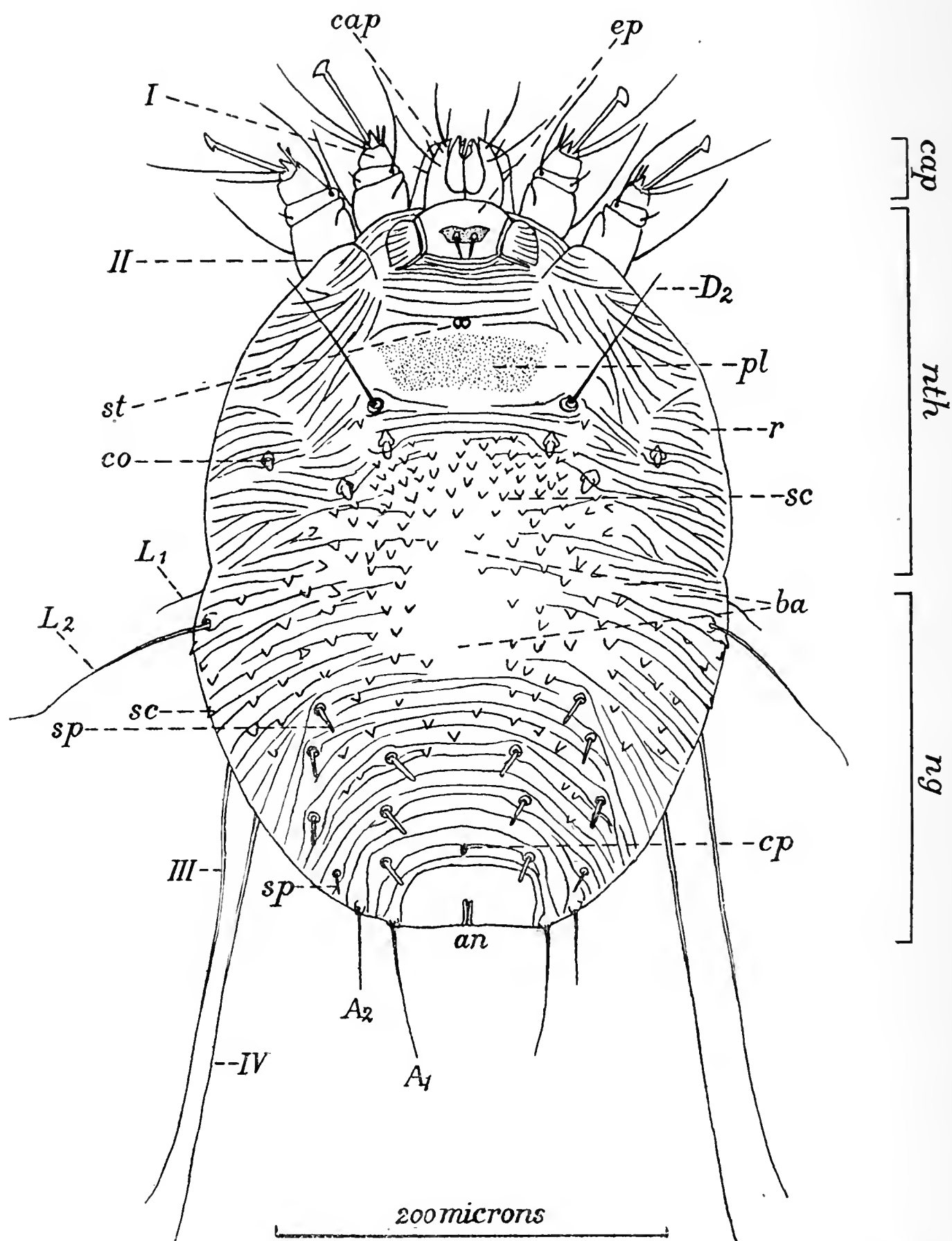


Fig. 1. *Sarcoptes scabiei* var. *equi*, dorsal surface of adult female: *A* 1, 2, anal setae; *an*, anus; *ba*, bare area; *cap*, capitulum; *co*, cone; *cp*, copulatory papilla; *D* 2, second dorsal seta; *ep*, epistome; *L* 1, 2, lateral setae; *ng*, notogaster; *nth*, notothorax; *pl*, plastron; *r*, integumentary ridges; *sc*, scale; *sp*, spine; *st*, ring-shaped rudiment of stigma; *I*–*IV*, legs. $\times 220$.

translucent whitish colour, except where more strongly chitinized parts which lie on or near the surface are apparent by reason of their brown colour. The surface of the mite is covered with fine parallel ridges. One may generally

detect a division of the body into an anterior notothorax (*nth*), and a posterior notogaster (*ng*). The line which divides these parts of the body is slightly in front of the middle and runs across the ventral surface behind the tocostome;

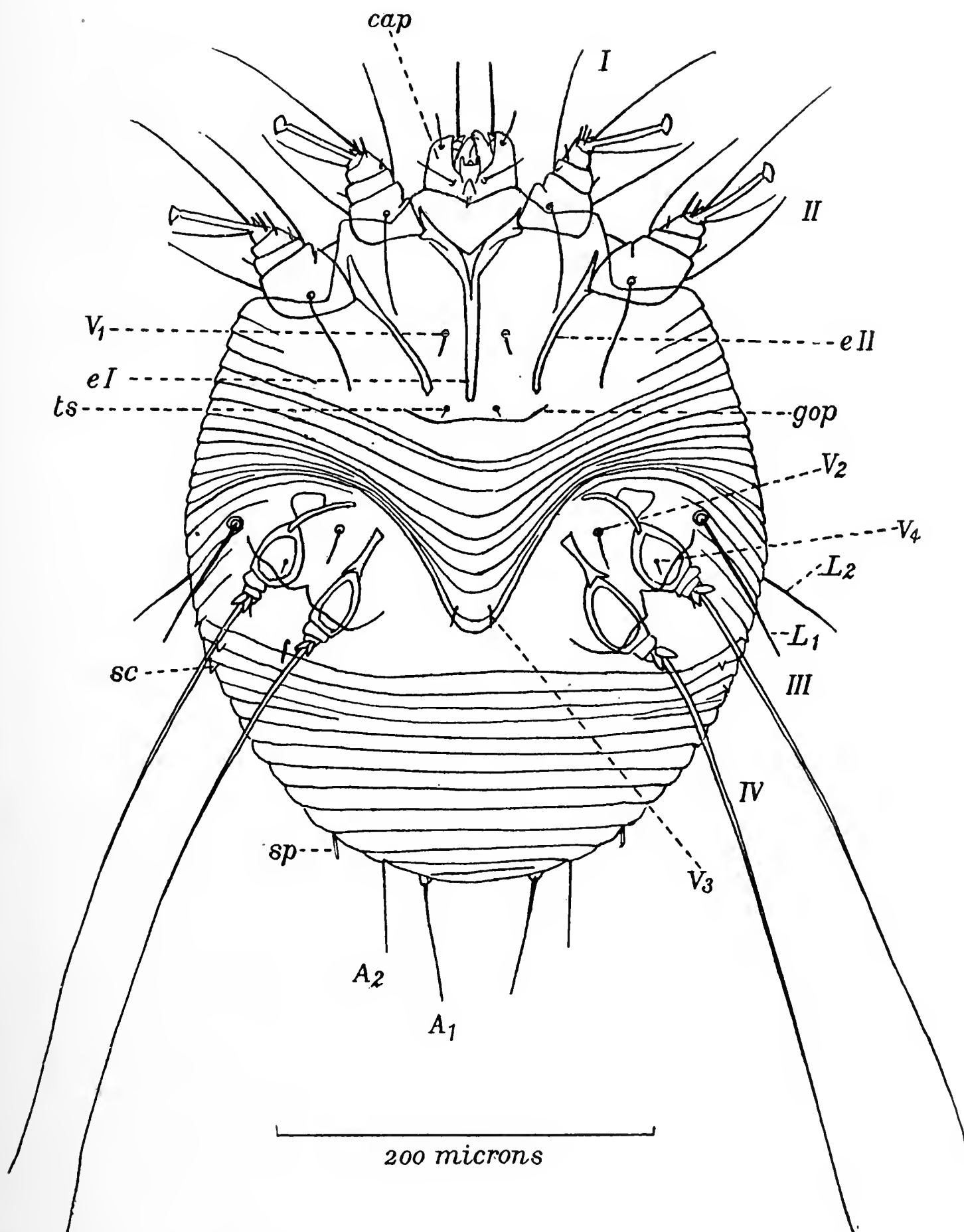


Fig. 2. *S. scabiei* var. *equi*, ventral surface of adult female. *A* 1, 2, anal setae; *cap*, capitulum; *e* I–*e* II, epimeres of legs; *gop*, genital operculum; *L* 1, 2, lateral setae; *sc*, scale; *sp*, spine; *ts*, tocostomal seta; *V* 1–4, ventral setae; *I*–*IV*, legs. $\times 220$.

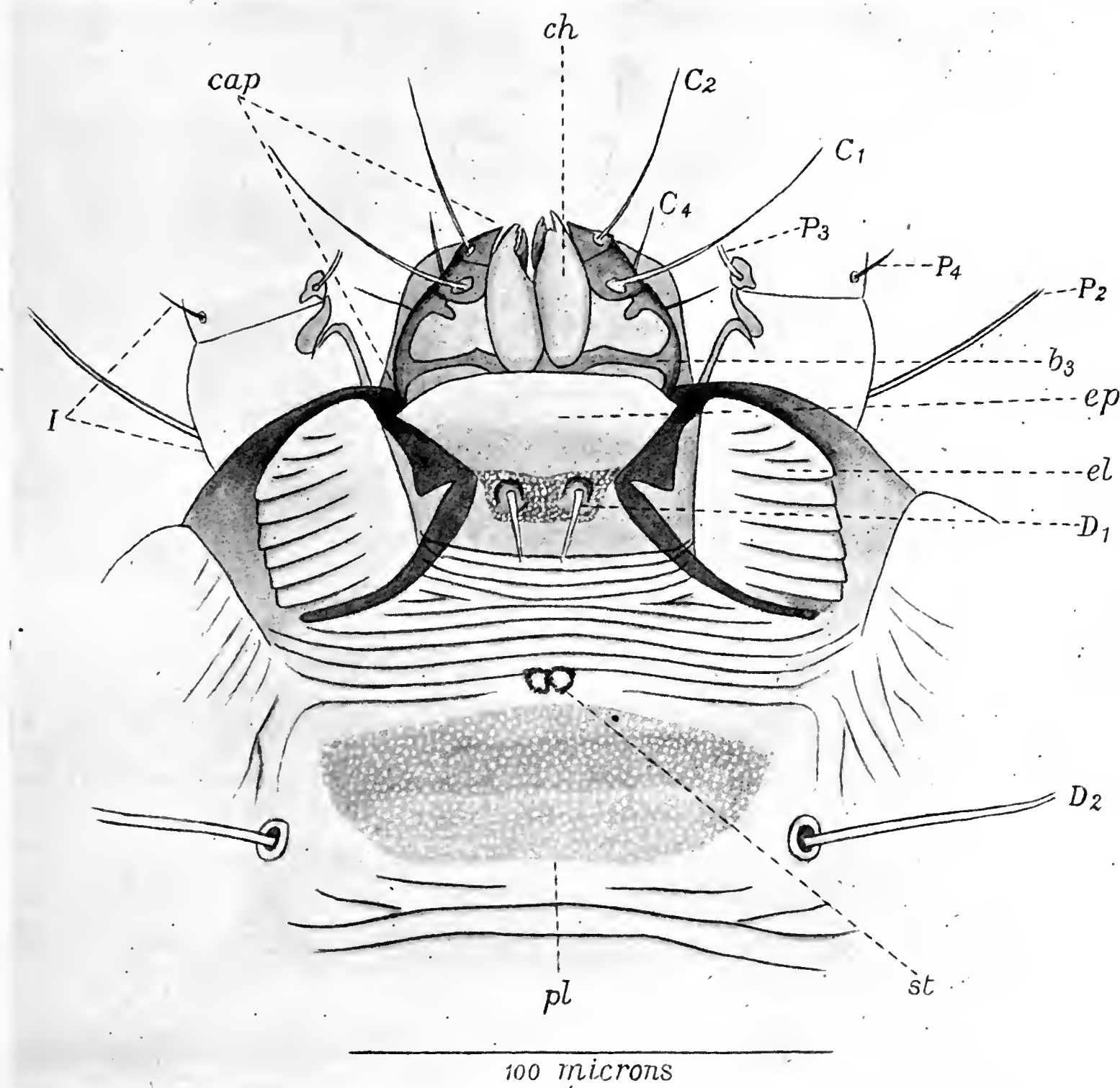
but its position is so indefinite that I shall endeavour to avoid the use of the terms notothorax and notogaster as far as possible. In living specimens in an advanced stage of pregnancy, when the egg has come forward to the tocostome,

the furrow between the notothorax and notogaster may be obliterated. It was from a specimen in this condition that Fig. 2 was drawn. The terms cephalothorax and abdomen which have been used for these two portions of the body are unsuitable because as Warburton says (*l.c.* p. 272) they bear a different meaning when applied to other groups of Arachnoidea; I might add that application of these terms to any Arachnoidea is open to objection.

THE DORSAL SURFACE.

The dorsal surface (Fig. 1) is covered with fine parallel ridges which generally run transversely except where they follow the lateral contours of the body, or bend partially round the anus. A fold of the dorsal integument, the epistome (Plate VII, *ep*), covers the insertion of the basis capituli into the camerostome. The camerostome is a hollow in the front of the body for the reception of the capitulum. It cannot be seen from above. The epistome, which is in fact the dorsal wall of the camerostome, is not transversely ridged. Across its posterior part stretches a small area covered with shagreened sculpturing and containing two relatively large pits from which the setae (*D 1*) arise. This small shagreened area was noticed by Railliet in 1887 in *Sarcoptes laevis*, which is now placed in the genus *Cnemidocoptes* Fürstb., but it has not I think been described in any *Sarcoptes* in the restricted sense. Lobes of integument similar to the epistome cover the bases of the first and second legs; that covering the base of the first leg is particularly prominent, for the dorsal epimere surrounds a raised portion of the integument, the epaulette (*el*). There is no dorsal epimere and no epaulette in relation to the second leg. The ridges into which the greater part of the integument is thrown are faint and often interrupted in the mid-dorsal region over the greater part of the notogaster (see p. 119); they are absent from a small area on either side of the anus, and from a conspicuous roughly rectangular area on the notothorax. This area is the plastron of most authors including Railliet; the rugose area of Munro. (The "plastron" of Robin is on the ventral surface; it is the place of union of the first pair of epimeres with the sternum.)

The shape of this plastron (*pl*), as I shall call it, is shown on Plate VII. Its width is about $2\frac{1}{2}$ times its extent from before backward; the anterior margin is convex, the posterior straight; the anterior angles are acute, the posterior obtusely rounded. It is extremely difficult to represent this structure in a drawing, but I have endeavoured to do so in Plate VII. In Fig. 1 I have stippled it, so as to show its extent, but have not been able to indicate that the whole surface is shagreened; under an oil-immersion lens the appearance is as of a great number of points of light, regularly arranged upon a yellowish background. It is very slightly more strongly chitinized, and slightly browner in colour than the rest of the integument, but it is the easiest thing possible to overemphasize its degree of chitinization in drawing it. In fact the very word plastron is unsuitable, because it makes one think of an impenetrable plate of mail, but it is at any rate to be preferred to "rugose area," a term which is



Sarcoptes scabiei var. *equi*, anterior end of dorsal surface of adult female. *cap*, capitulum; *ch*, chelicera; *C* 1-4, setae of capitulum; *D* 1-2, dorsal setae; *el*, epaulette; *ep*, epistome; *P* 2-4, pedal setae; *pl*, plas-tron; *st*, rudiment of stigma (?); *I*, first leg. $\times 540$.

The plate and all the text-figures have been drawn with a camera lucida.

definitely misleading, because the area is not rugose. Just anterior to the plastron there are two ring-shaped markings (*st*). These lie beneath the integument and are crossed by one of its folds. Their nature is entirely obscure. They consist of chitin and under high magnifications it is seen that their outline is slightly irregular. Mégnin described them as rudiments of stigmata; Munro figured them but did not mention them in his text; other authors, except Warburton, appear to have failed to notice them.

Behind the plastron half a dozen ridges pass across the dorsum and behind these there are a number of transverse rows of scales (*sc*, Figs. 1 and 3). These scales cover the greater part of the mid-dorsal surface; they are absent from the flanks and from the area in front of the anus. The rows on which they are arranged are continuous laterally with the ordinary ridges of the integument, and the scales represent a specialization of the ridge. Various stages in the perfection of the scale may be seen, from short blunt projections to the fully-developed organ, which is not thickly chitinized nor darker in colour than the integument from which it springs. In shape the scale is nearly equilateral, with a slightly concave base and very slightly convex sides;

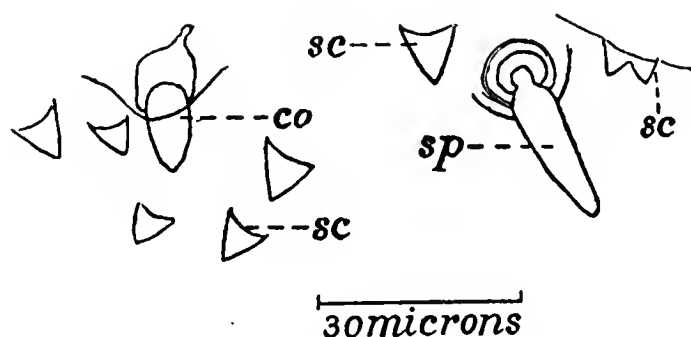


Fig. 3. *Sarcoptes scabiei* var. *equi*. On left, cones and scales from the notothorax; on right, scales and spines from the notogaster of an adult female; *co*, cone; *sc*, scale; *sp*, spine.

it is never acorn-shaped. It is difficult to count the scales owing to their irregular arrangement, but they number a little over one hundred. In Fig. 1 I have drawn an example in which the transverse rows are relatively regular, and in which traces of a longitudinal arrangement can be seen. The precise direction in which some of the smaller scales point is also variable, though the general direction of all is backwards. The largest scales are those on the lateral part of the notogaster.

In the example figured in Fig. 1 there is a single definite bare area (*ba*) in the middle of the dorsum. Its margins are ill-defined but from it scales and ridges are absent. In other adult females taken from the same horse this bare area is divided into two parts by a row or two of small scales passing transversely across it. The fact that this character is variable is of some importance because Railliet and also Canestrini and Kramer regard the presence of two bare areas as a specific character of *Sarcoptes scabiei* var. *equi*. As there is also variation in the number of scales present and in the regularity of the rows in which they are set I think we are justified in deciding that specific characters founded on them are of doubtful value. It is to these bare areas which occur

among the scales covering the notogaster that Railliet gave the name "clairière" in distinction to "plastron," by which he denotes what we call the plastron or rugose area. He remarks (1895, p. 641) with reference to *S. scabiei* Latr. that: "les écailles dorsales, chez la femelle ovigère, tendent en général à s'atrophier sur deux points, en laissant vers la ligne médiane une clairière plus ou moins nette; au niveau des épines postérieures du notothorax (clairière antérieure), et au niveau des épines antérieures du notogastre (clairière postérieure)."

It is probably to these areas that Canestrini and Kramer refer when they define *S. scabiei* var. *equi* as having "eine vordere und eine hintere Rückenblosse." Warburton is, I believe, mistaken when he states that "clairière," "blosse," and "plastron" have all been used for the same structure, the plastron on the notothorax.

Behind the plastron on each side are three cones (*co*), ("notothoracic cones," Munro, Warburton; "Schulterzapfen," Canestrini and Kramer; "épines courts," Railliet; "coni scapolari," Canestrini).

The antero-median cone lies behind the posterior angle of the plastron, the antero-lateral far out on the side of the body, and the posterior nearer the first than the second. The cone is considerably larger than the spine, acorn-shaped, covered at its base by one of the ordinary ridges of the integument, and articulated into a depression in the centre of an oval chitinous plate; this plate lies beneath the integument but is visible by reason of its brown colour.

On each side of the posterior part of the body there are seven blunt, lancet-shaped spines (*sp*). They are arranged in two longitudinal rows, a straight median one of three spines, and a curved lateral of four. It is convenient to designate this arrangement as 3 : 4. The posterior spine of the lateral row is finer and shorter than the rest. The spines are essentially similar to the cones and differ only in being articulated to a round plate of chitin, which appears to lie in, not beneath, the integument. The cones and spines differ from the scales in that instead of being merely specialized portions of the integumentary ridges, they interrupt the ridges and arise from independent chitinous plates; they are possibly specialized setae, and a consideration of the chaetotaxy of allied genera might show whether this is so.

The chaetotaxy is of systematic importance, and there is considerable disagreement between the figures of earlier writers; some of the smaller setae for long passed unnoticed, and not seldom ventral setae were observed by transference and figured on both dorsal and ventral aspects. I have lettered every seta, and this will help with the comparative study of other species of the genus. Robin (1860) has already devoted attention to the comparative chaetotaxy.

On the dorsal surface there is a pair of short thick bristles (*D 1*) arising from pits in the small shagreened area which lies just in front of the epistome. Behind the posterior angle of the plastron a pair of very long setae (*D 2*) arise; these setae are directed outwards and forwards in the living mite when

it is removed from its burrow, but it is difficult to believe that they point in this direction when the mite is actually burrowing. On the side of the body there is a fairly long lateral seta (*L* 2) situated on a level with the posterior bare area. Warburton (*l.c.* p. 273) says that this is the "tasthaar" of Fürstenberg, but that author states (p. 181) that he uses this term to include all the larger setae of the body and legs. This seta differs from all others in that its origin is from a short but definite papilla. The base of the seta *L* 2 is latero-ventral, but the tip can be seen from above. On each side of the anus are two anal bristles; the median pair *A* 1 are longer and stouter than the lateral pair *A* 2; the median pair are strictly terminal, the lateral, distinctly on the dorsal surface, close to the edge of the body. It is difficult to say which are Munro's first pair of notothoracic bristles; if "they lie just below the camero-stome" it is not easy to see why he attributes them to the dorsal surface at all.

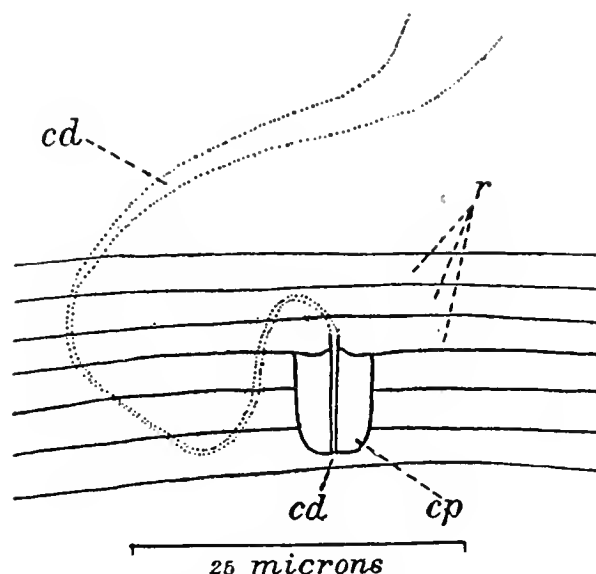


Fig. 4. *Sarcoptes scabiei* var. *equi*. Copulatory papilla of an adult female; the copulatory duct is visible through the integument and is shown with a dotted line; *cd*, copulatory duct; *cp*, copulatory papilla; *r*, ridges on the integument.

Immediately in front of the anus is an extremely minute papilla perforated by a fine tube which opens upon its summit. The papilla (*cp*, Fig. 4) itself is scale-like, flat and very freely moveable. The tube or copulatory duct (*cd*) passes from the summit of the papilla through its base, and penetrates deeply into the body. It appears to have a chitinous lining and can be traced for some distance, and it can be seen that it becomes gradually wider and wider. I shall return to a discussion of its function when I describe the male genitalia (p. 138), and the immature female (p. 139).

The papilla was first observed so long ago as 1861 by Gudden, who wrote an exceedingly interesting paper, dealing among other things with the internal and external genital organs of *Tyroglyphus* and *Sarcoptes*. He chose *Tyroglyphus* by reason of its larger size, and when he had familiarized himself with its internal anatomy he turned to *Sarcoptes*. He figures the whole genital tract of an adult female; it consists of the papilla, as I have described it, a narrow tube leading from it to a spherical spermatheca, and two divergent ovarian tubes which pass from the spermatheca to unite immediately behind

the tocostome or orifice by which they discharge the eggs to the exterior; at their point of union there is a lubricating gland. Gudden's results must have been due to pure dissection without the aid of the microtome, and are nothing less than astounding. I have made no attempt so far to investigate the internal anatomy of *Sarcoptes*, and I only find myself in disagreement with Gudden on one point; he figures the copulatory duct or vagina as a fine tube of even calibre passing from the copulatory papilla to the spermatheca; I find that it widens gradually and that this is the case in every specimen examined. Gudden's other results I accept with all humility. A year before the publication of Gudden's work the papilla had been seen and figured by Robin (1860); unfortunately he regarded it as a median spine, similar to the other notogastric spines except for its smaller size. On the whole Gudden's discovery has received very little attention though it is quoted in Railliet's text-book (1895). Mégnin (*a*) copies one of Robin's figures which show the papilla as a spine, though he makes no acknowledgement of the source of the figure and (1886) states that "les Glyciphages sont les premiers acarïens chez lesquels nous constatons l'existence d'un organe spéciale de copulation; chez les autres Sarcoptides, la copulation se fait par la fente anale, comme nous avons maintes fois constaté." Trouessart (1893) corrects this error, and says that his own observations support Gudden; he states that the male copulates with the immature female, and that it is in this stage that one best sees "la poche copulatrice"; the opening of the "poche," or as I should call it the papilla, looks like a hair-base from which the hair has been broken, but it is unpaired and median; the papilla is always referred to as being *behind* the anus, a misinterpretation which we must account for by supposing that in compressing specimens for microscopic examination the anus was brought round to the ventral surface. Munro (1919) and Warburton (1920) make no reference to the papilla nor to the discrepancy between Mégnin's views and the facts of the case; it appears that they overlooked the papers of Gudden (1861) and Robin (1860).

The anus (*an*) is a longitudinal slit at the extreme end of the body; it extends slightly more on the dorsal surface than on the ventral which is interesting because in the closely allied genera *Notoedres* and *Prosopodectes* it is distinctly dorsal in position. The anus is bounded on each side by a straight, thick lip. Fürstenberg figures the anus of various species of *Sarcoptes* not as a straight slit, but with a concavity towards one side or the other. This at any rate so far as *S. scabiei* var. *equi* is concerned is an error, produced probably by the study of slightly compressed specimens. Fürstenberg was on the whole a most careful man, but it is important to correct his errors because his figures have been reproduced widely. Robin (1860) and Railliet (1895) figure and describe it correctly.

THE VENTRAL SURFACE.

The under surface of the body (Fig. 2) is nearly flat, and is crossed normally by the indefinite sulcus which divides the notothorax from the notogaster. It is covered by ridges of integument, transverse for the most part, but sweeping round the articulation of the posterior legs so as to become longitudinal; where the integument is strengthened by the ventral epimeres it is not thrown into ridges. At the anterior end of the ventral surface there is a deep V-shaped indentation. The sides of the V are the lower margin of the camerostome, and pass round the basis capituli somewhat like a collar. There are similar V-shaped indentations for the reception of the bases of the legs of the first two pairs. The ventral epimeres (*e I-IV*), are the most conspicuous objects on the ventral surface of the mite. In sections of the whole organism they appear as thickenings of its chitinous integument, extending into the body as ridges. They are to be regarded as specialized parts of the exoskeleton of the body, and not as parts of the leg; their function is to provide a firm *point d'appui* for the leg, and this they do by stiffening the exoskeleton which is elsewhere so soft. Their distal end is forked to receive the obtusely angular articular surface of the corresponding leg. One is tempted to think of them as ridges for the origin of muscles, as apodemes in fact; I am not prepared to say that they never fulfil this function, but it is certainly not their prime function. The epimere of the first leg (*e I*) passes backwards and inwards to meet its fellow of the opposite side at a point slightly behind the insertion of the basis capituli into the camerostome. From their point of union they pass backwards as a single chitinous structure, which terminates bluntly in front of the genital operculum (*gop*). This median structure, the product of the fusion of the first pair of epimeres, is the sternum of most authors, "sternal bar," and "sternal rod" of Warburton, "*pièce commune ou sternale*" of Robin. The name "plastron" is given by Robin (1860) to the point at which the first pair of epimeres unite.

The epimeres of the second pair of legs (*e II*), resemble those of the first pair in size, but differ in the greater development of the mesial limb of the fork which receives the articular surface of the basal joint of the leg; this limb of the fork is prolonged for a considerable distance up the space which separates the first and second legs (Fig. 8). The shaft of the epimere is curved towards the middle line, so that its basal part is nearly parallel to the sternum. The basal (posterior) end of the epimere is shown in Fig. 5. It bears a minute point and shows a very slight tendency to being forked. The epimeres of the third and fourth pairs of legs differ from the anterior ones in being much shorter. That of the third leg is curved so that its proximal end approaches the fourth. On its lateral side it is prolonged out into a thin semi-circular flange, the free edge of which gradually passes into the chitin which covers the body. The fourth epimere is short, stout and nearly straight; it is shallowly forked at its proximal end, and more deeply distally, where it articulates with the fourth leg.

The following setae are present on the ventral surface: a pair of short setae (*V 1*) on either side of the sternum; a very short pair, the tocostomal setae (*ts*), close to the middle line on the base of the genital operculum; a pair of rather stout but short setae (*V 2*) arising from the bare space between the epimeres of the third and fourth legs; a pair of extremely fine setae inserted close to the middle line among the integumentary ridges which lie between the bases of the last pair of legs (*V 3*); and a short seta (*V 4*) on the cuff of integument which covers the base of the third leg.

In addition to these there are in the ventro-lateral region the setae *L 1*, which arise anterior to the dorso-lateral pair (*L 2*), and on a level with the epimere of the third leg. The seta *L 1* is longer than any of the ventral setae but not so long as *L 2*. Other setae, which are visible on the ventral surface, but belong to the chaetotaxy of the legs are described on p. 130. The median

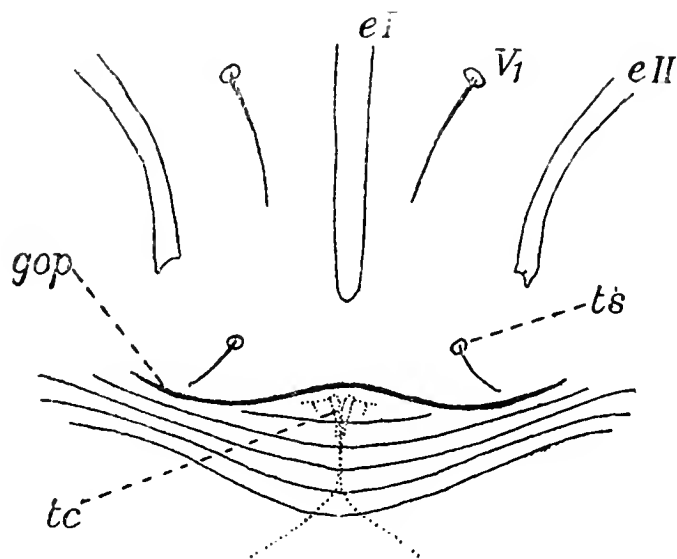


Fig. 5

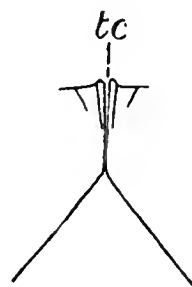


Fig. 6

Fig. 5. *Sarcoptes scabiei* var. *equi*. Ventral view of the genital operculum and surrounding parts in an adult female. *e I*, *e II*, epimeres of first and second legs; *gop*, genital operculum; *tc*, tocostome; *ts*, tocostomal seta; *V 1*, first ventral seta.

Fig. 6. Ventral view of actual tocostomal orifice; the same structure as shown in dotted line in Fig. 5. *tc*, tocostome.

tocostomal seta which is figured by Fürstenberg and others, and apparently by Munro (1919), does not exist, at any rate in *S. scabiei* var. *equi*. As I shall explain below these authors have seen the longitudinal aperture of the tocostome, and figured it as a seta. All the setae on the ventral surface are directed backwards.

Three or four scales (*sc*) can generally be seen from below, on the flank at the level of the fourth pair of legs. The tip of the last spine (*sp*) of the outer row is also often in sight, projecting over the posterior end of the body, but it is of course a dorsal structure. With these exceptions there are no scales or spines on the ventral surface.

Behind the epimeres of the first and second pairs of legs a wide transverse flap, very shallow from before backwards, can easily be seen. This is the genital operculum (*gop*, Fig. 5), bearing at its base the tocostomal setae (*ts*).

This operculum¹ covers the actual orifice of the tocostome (*tc*) ("vulve," Robin; "vulve de ponte," Neumann), a longitudinal slit between two closely opposed slightly thickened lips. These lips lie slightly beneath the integument of the body, and are shown most clearly in Fig. 6. They are somewhat thickened in front where they are opposed to one another; further back they are more weakly chitinized and may be seen to diverge as they pass deeply into the body. In specimens which have been killed slightly before oviposition the lips may be seen widely separated to allow for the passage of the egg. There is therefore no doubt at all that this orifice is the tocostome, that is to say the birth opening or passage through which the egg is laid.

The earliest and at the same time the most accurate extant figures of the genital operculum are those of Robin (1860) and Gudden (1861). Since that time it has been figured and described by Delafond and Bourguignon (1862), and by Railliet (1895). Other authors seem to have failed to see it, and no one so far as I am aware has described the actual longitudinal orifice of the tocostome, except Gudden (1861). Railliet, for instance, speaks of "une fente transversale," obviously the line of opening behind the genital operculum. It is an extraordinary thing that a writer so recent as Munro (1919) failed to observe the genital operculum, and figures only a median structure which might be taken to represent either the opening of the tocostome, or the (non-existent) median seta first figured by Fürstenberg (1861).

THE CAPITULUM.

The structures which comprise the capitulum of *Sarcoptes* have been interpreted in a different way by almost everyone who has seriously tried to describe them. Modern technique and a modern microscope have helped me to produce the following description, which I believe is an improvement upon those previously published. I propose first to describe the structure of the capitulum and its component organs, and then to discuss certain points of interpretation and homology.

The capitulum (*cap*) consists of the chelicerae (*ch*) and palps (pedipalps) (*pp*), and the basis capituli or chitinous capsule to which they are attached. The general shape of the capitulum resembles that of a thumb-nail, as can be seen from Plate VII and Fig. 7; its length is about 60μ , breadth 55, and depth from the dorsal to the ventral surface 50–60 at the base. Seen from the side the dorsal surface is flat, the ventral convex, and these approach one another from base to apex. The insertion of the basis capituli into the camerostome has already been described; the base of the capitulum is overlapped by the epistome above, and partly covered by the diverging limbs of the V-shaped opening below. The structure of the basis capituli, which is the name given to the whole capitulum except the appendages, is as follows. The chitinous

¹ The tocostome is generally overlapped by the margin of the genital operculum. In the example which I have chosen for figuring (Figs. 5 and 6) it lies slightly posterior to the margin of the operculum and is therefore more clearly seen.

investment of the basal part of the basis capituli is in some places thickened into bands which act as mechanical stays. The exact disposition of the bands on the ventral surface can be seen in Fig. 10. They support the base (*b 1*) and sides (*b 2*) of the organ; at its posterior extremity the bands *b 1* unite at the middle line to form a rounded mass of chitin (*z*), the "menton" of Robin. The first epimere (*e I*) is in close relation to the side (*b 2*) of the basis capituli, but there is no articulation between them. Similar bands pass from the sides towards the middle line on the dorsal surface (*b 3*, Plate VII), and also on the

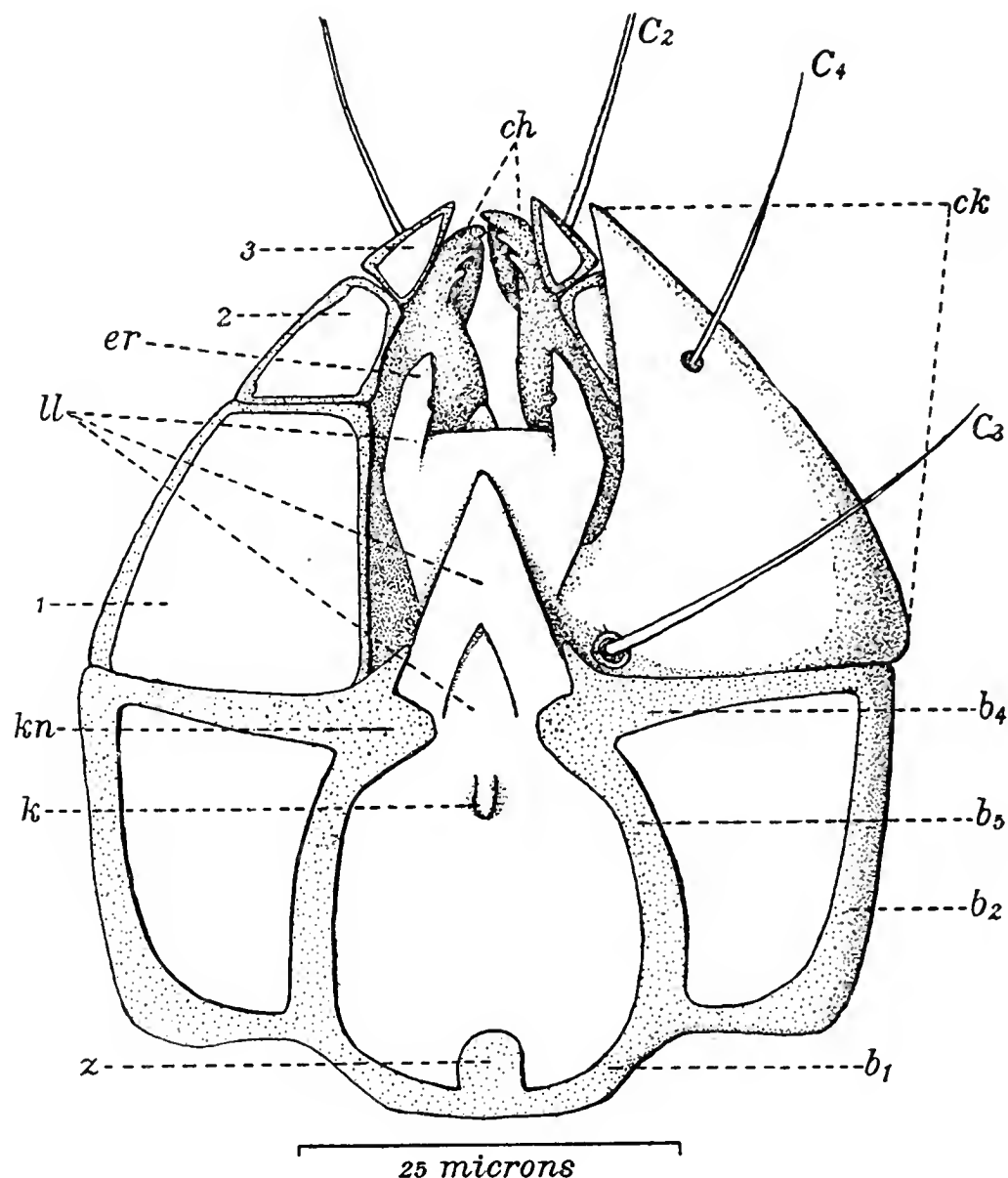


Fig. 7. *Sarcoptes scabiei* var. *equi*. Capitulum of adult female seen from below after removal from camerostome. *b 1*, *2*, *3*, *4*, bands of chitin forming supports for the basis capituli; *C 2-4*, capitular setae; *ch*, chelicera; *ck*, cheek-piece; *k*, keel; *kn*, knob of chitin, the maxilla of Robin; *ll*, lower lip; *z*, part of the thicker chitinous skeleton of the basis capituli, the "menton" of Robin.

ventral (*b 4*). The inner end of *b 4* meets the longitudinal *b 5* to form a rounded heavily chitinized knob (*kn*). On the mid-ventral line there is a minute keel (*k*) projecting downwards.

The transverse bands *b 3* on the dorsal, *b 4* on the ventral side, divide the basal third of the basis capituli from the rest. From *b 4* springs the cheek-piece (*ck*), a triangular plate of the thinnest and most colourless chitin, which covers the palp ventrally and laterally. This cheek-piece is so transparent that one has difficulty in seeing it when it is folded close about the palp. In

mounted specimens, especially those which have been exposed to some pressure, it is frequently pushed to the side; it then becomes more easily seen. The cheek-piece bears two setae on its ventral surface, *C* 3 a large stiff seta arising from a conspicuous pit near the mid-ventral line, and *C* 4, shorter and finer, arising close to the lateral margin of the cheek-piece near its extremity.

Between the cheek-pieces on the ventral surface is a complex structure which I prefer to call the lower lip (*ll*). It is the homologue of what is called the hypostome in ticks, but the term is slightly ambiguous. In the Ixodea it is defined as a "median ventral structure arising from the basis capituli" (Nuttall, Warburton, Cooper and Robinson, 1911, p. 127), but in the rest of the Acarina it is used (*e.g.* by Hirst) for a ventral structure comparable to the epistome; in fact in this sense of the word the epistome and

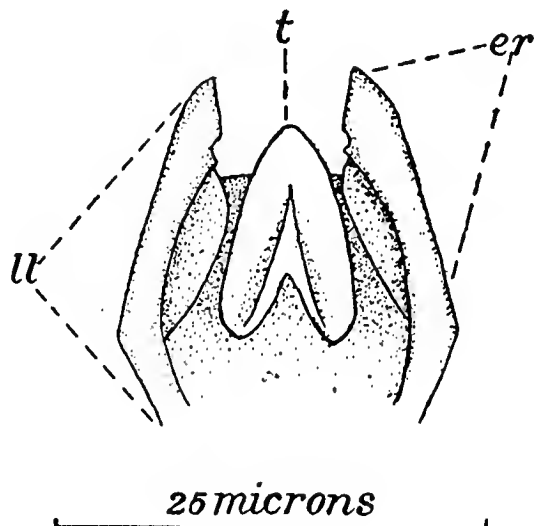


Fig. 8

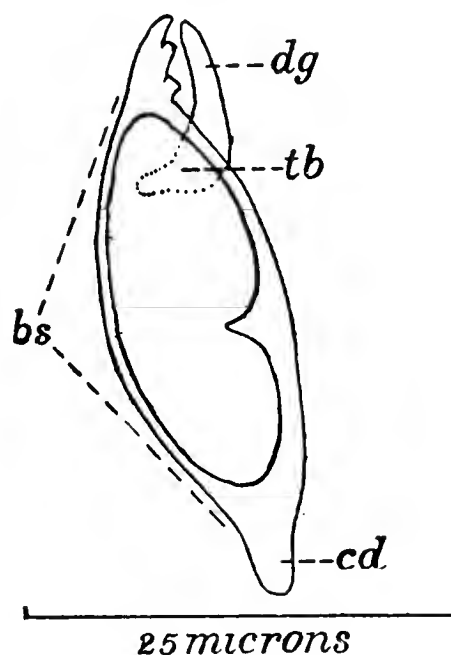


Fig. 9

Fig. 8. *Sarcoptes scabiei* var. *equi*. Dorsal aspect of lower lip of adult female. *er*, erect body; *ll*, lower lip; *t*, languette or tongue.

Fig. 9. Left chelicera, lateral aspect. The dorsal side is to the left. *bs*, base of chelicera; *cd*, condyle; *dg*, digit; *tb*, tubercle of digit.

hypostome are folds of the general body integument and lie respectively above and below the insertion of the basis capituli into the camerostome. The lower lip as I shall call this structure (Fig. 8), arises from the rounded knobs (*kn*) of chitin which as I have already said are formed by the union of the chitinous bands *b* 4 and *b* 5. It is a flat strip of chitin, for the most part thin and colourless, strengthened at its base by an acutely angled area of slightly thicker chitin. At each side of its extremity arises an erect body (*er*), which is immediately ventral to the chelicerae. These erect bodies are not articulated upon the extremity of the lower lip, but are simply continuations of its lateral margin; they are sharply excavated on their mesial aspects. Between these erect bodies the extremity of the lower lip is abruptly truncated. The tip of the structure known as the languette or tongue (*t*) lies upon the dorsal side

of the extremity of the lower lip, and may be seen projecting over its truncated margin. The appearance of the languette from above is seen in Fig. 8. It is a flat oval piece of chitin, deeply incised at the base, and thickened by a chitinous median ridge. It is analogous, if not homologous, to the hypopharynx of insects.

The palp (*pp*) (morphologically the pedipalp) is an organ lying on each side of the capitulum above the cheek-piece, below and to the side of the chelicera. In general shape it is cultriform, and when the two palps are flexed their terminal joints meet across the chelicerae. The palp is articulated to the base of the basis capituli over a wide area along the line of the chitinous band *b* 4; it consists of three joints, and the lines dividing them from one another are more clearly marked below than above. The first joint must be nearly immovable, by reason of its articulation over a wide base to the basis capituli, and of its position between the chelicera above and the cheek-piece below; it is greater in length than the second and third joints combined, and is devoid of setae. The second joint bears the first capitular seta (*C* 1) on its dorsal surface; the third joint which is the shortest of the three and triangular in shape, bears the seta *C* 2 on its dorsal surface. The third joint terminates in a blunt point, without any of the delicate finger-like processes found in the closely allied *Psoroptes* (Buxton, 1920).

The chelicerae (Fig. 9) lie on the dorsal side of the capitulum, and are exposed for the greater part of their length. They lie above the palp and close to the middle line. Their general shape can be seen from Fig. 9. The chelicera is short; thick, slightly concave downwards and convex upwards. It consists of two portions, a base (*bs*) and a digit (*dg*). The base which forms the main part of the chelicera, bears a rounded articular surface or condyle (*cd*), which is in contact with a conspicuous articular surface inside the basis capituli. At its extremity it is decurved so as to form the upper limb of a chela, and it bears three teeth. To the mesio-inferior aspect of the base is articulated the digit (*dg*), which is not toothed but can be brought into opposition with the toothed extremity of the base; this completes the chela. All previous authors, except Robin, who give any attention to the matter figure and describe the digit as toothed. This is I believe due to an optical illusion; the whole chelicera is very small and when the chela is closed the teeth at the extremity of the base overlap the margin of the digit and impart to it a toothed appearance. The two parts of the chelicera are so jointed together that the tubercle of the digit lies free in the interior of the base. A muscle inserted on the tubercle and arising from the apodeme in the ventral side of the base would close the chela by bringing the two portions together. I have never been able to see such a muscle either in *Sarcoptes* or in *Psoroptes* in which genus I have recently figured a similar disposition of tubercle and apodeme.

So much for the description of the capitulum. There remain to be considered certain discrepancies between my interpretation and those of earlier writers.

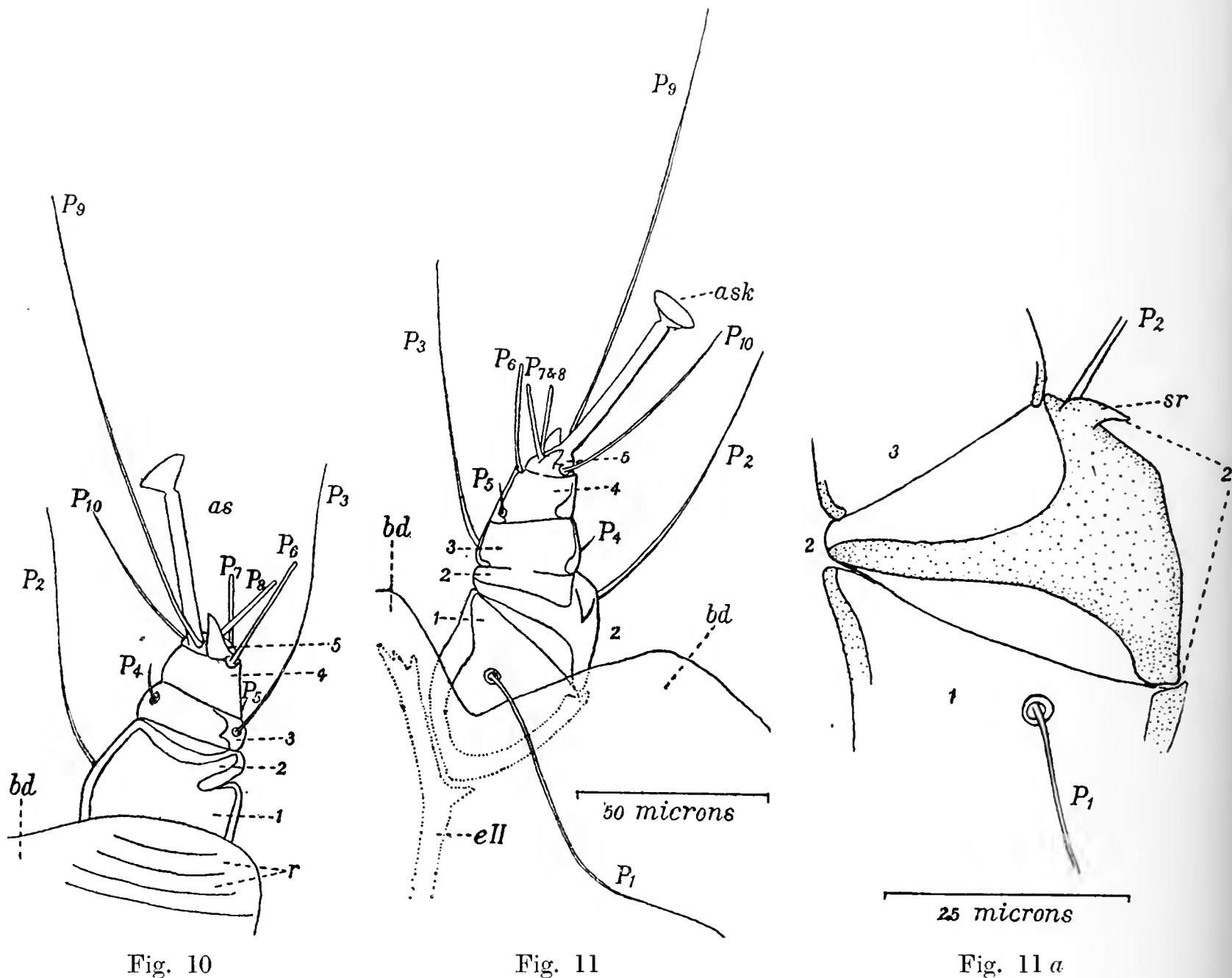
Robin's drawings of the mouthparts, etc., of *Sarcoptes* are better than those of any of his predecessors or successors, but he made serious mistakes in interpretation. It appears that he was too much inclined to read a knowledge of insect anatomy into his observations on *Sarcoptes*. The result is that he regarded the pedipalps (or "palps" in the acarological but not entomological sense) as the homologues of the maxillary palp of insects; these pedipalps, or "palps" are segmental appendages in the strict sense of the word and therefore comparable to the whole of an insect appendage. Having made this mistake, a most natural mistake too in 1860, he searched for the rest of the "maxilla," and attached this name to the stout chitinous knob (*kn*) and the two chitinous bands (*b* 4, *b* 5) which unite to form it. In his definition of the Sarcoptidae he says (1860, p. 196): "à rostre pourvu de mâchoires inermes très petits portant des palpes maxillaires latéraux, volumineux, à trois articles"; he adds that the "mâchoires ou maxilles" are "soudées ensemble par la ligne mediane."

Robin's mistake was copied by Railliet, whose figure was copied by Neumann, whose description repeats the error. The mistake does not merely affect our idea of the structure of *Sarcoptes* for this supposed union of the second appendage to form a median unpaired organ has become one of the accepted characters of the Sarcoptidae, and is figured as such by Berlese (1912, p. 11) in a figure which shows diagrammatically the supposed differences in the structure of the capitulum in different families of Acarina. I submit that this is an important, if academic, point, and that none of these authors produce evidence that the median unpaired structure is part of the pedipalp, or of any other appendage. It appears better to regard it as formed from the basis capituli, and to admit that we know nothing of its derivation in the embryo. It is not generally considered that any median ventral part of the head of spiders or scorpions, or other Arachnoidea represents a part of a pair of fused appendages, and it is not necessary to believe that this is the case in the Acarina.

I find myself in disagreement with Robin on a second point, which is of minor importance. The cheek-pieces are so thin and colourless that their mere detection is a matter of difficulty. Robin figures them fairly accurately but insists that they are the lateral lips of the camerostome, in fact prolongations of the body integument comparable to the epistome; I have convinced myself that they do not spring from the body at all, but are a part of the basis capituli itself, a ventral laminar prolongation of it arising from the transverse bar (*b* 4), and passing forwards beneath the palps. Fürstenberg (1861) figured and described the cheek-piece, which he called "backe," and his figure is at any rate accurate in this point that he derives the cheek-piece from the basis capituli. He made, as is well known, the extraordinary mistake of providing all his *Sarcoptes* with two pairs of chelicerae; whether as Warburton suggests he once observed a *Sarcoptes* in process of moult and so became possessed by a fixed idea that there were four chelicerae, or whether he saw not only the extremity of the chelicerae but also of the erect bodies of the lower lip we do not know.

THE LEGS.

The anatomy of the anterior legs can be understood by reference to Figs. 10 and 11. The first and second legs are similar in all respects, as regards their size, chaetotaxy and their possession of five joints. More of the base of the leg can be seen from below than from above, owing to its ventro-lateral origin; the articulation of the first joint with the body is sunk in a pit, just as the



Figs. 10, 11 and 11 a. *Sarcoptes scabiei* var. *equi*. Second left leg of adult female. Fig. 10, dorsal, and Fig. 11, ventral aspects. Fig. 11 a, ventral aspect more highly magnified, the leg slightly rotated so as to show the spur on the second joint. *as*, stem of ambulacrum; *ask*, its sucker; *bd*, body; *e II*, epimere; *P 1-10*, pedal setae; *r*, integumentary ridges; *sr*, spur on second joint ("trochanteral spur"); 1-5, the five joints of the leg.

base of the capitulum is sunk in the camerostome. This point has apparently escaped notice hitherto, because it is difficult to detect the nearly transparent covering of integument with its wide V-shaped margin. In Fig. 11 a I have indicated with dotted lines the part of the first joint of the leg which is so covered. In Fig. 2 I have not dotted the corresponding lines, for with a low magnification one does not appreciate that these parts are covered at all. Actually of course they are extremely close to the surface. The first joint is

strengthened by a U-shaped, band-like thickening of its integument, the base of this U articulating with the fork at the distal extremity of the epimere, and the tip of each of its limbs with the second joint. On the ventral side of the joint a long seta (*P* 1) arises and passes backwards over the ventral surface of the body. The line of articulation between the first and second joints is oblique, and is apparent only on the under surface of the leg. On the upper surface the joints appear to be completely united, and it may be supposed that movement of the second article on the first is extremely limited; we must presume that there is some movement because as I have said there are definite articular surfaces between the ends of the U band of the first joint and the base of the second joint. On the mesial side of the leg there is an indentation, conspicuous from above and below, marking the line of the articulation between the first and second joints. On the under surface of the second joint (Fig. 11 *a*) is a T-shaped piece of chitin, the base of which articulates with the first joint; the head of the T lies upon the lateral margin of the leg; from it arise two structures, the seta *P* 2 which reaches as far as the tip of the ambulacrum, and the spur (*sr*), first described by Mégnin. This is a point of some importance for no writer since Mégnin's time, with the sole exception of Canestrini, has figured or described this structure, and some doubt has arisen as to whether it actually exists. Mégnin first observed it in *Sarcoptes* taken from the horse, and on this character and others he founded a new species *S. uncinatus*; when, however, he looked for the spur in *Sarcoptes* from other hosts he invariably succeeded in finding it. It is probably present throughout the genus, for Canestrini figures it (1894) in the species *dromedarii*, *leonis*, and *precox*.

The third joint is short, and on each side of it there is a longitudinal band-like thickening of the chitin. From each of these thickenings springs a seta; on the median side *P* 3 arises from a pit in a small chitinous plate, and on the lateral side *P* 4, a very short, stiff seta; both of these are on the upper side of the leg. The fourth joint is longer than the third, which it resembles in being stiffened by a mesial and a lateral region of relatively thick chitin. The mesial side of the joint is noticeably straighter than that of any other joint; from its base on the ventral side arises the minute seta *P* 5; the extremity of the joint is hollowed out; from the excavation arises *P* 6, a short stiff seta which does not taper to a point but is equally thick throughout its length. The fifth joint is short and may barely be seen among the multitude of structures which arise from it. The largest of these is the ambulacrum arising directly from the ventral side of the joint. This ambulacrum consists of a cylindrical chitinous stem (*as*), which suddenly narrows at its distal extremity where a cup-shaped sucker (*ask*) is attached to it by a narrow base. Fürstenberg figured a fine tube passing through the stem and opening in the concavity of the sucker. I believe that no such tube exists and that the error is due to an optical illusion such as is easily produced when one looks at a solid transparent cylinder, and I have confirmed my opinion by examining transverse sections

of the ambulacral stem, which proves to be a solid rod. The minute seta figured by Robin springing from the extremity of the ambulacral stem does not I believe exist. The ambulacrum as a whole is the "haftnapf" of Canestrini and Kramer.

The terminal joint of the leg is also armed with two claws. These arise from the proximal part of the joint, the one on the dorsal surface, the other on the ventral surface. Their shape can be seen in Figs. 7 and 8. Four setae also arise from this small and overcrowded joint. The first two (*P* 7 and *P* 8) are on the mesial aspect of the joint, close to the stiff seta (*P* 6), similar to it in shape, and slightly shorter. The third (*P* 9) arises close to the base of the ambulacral stem on the dorsal side of the leg; it is finely tapered and more than twice as long as the ambulacrum. The third (*P* 10) arises from the

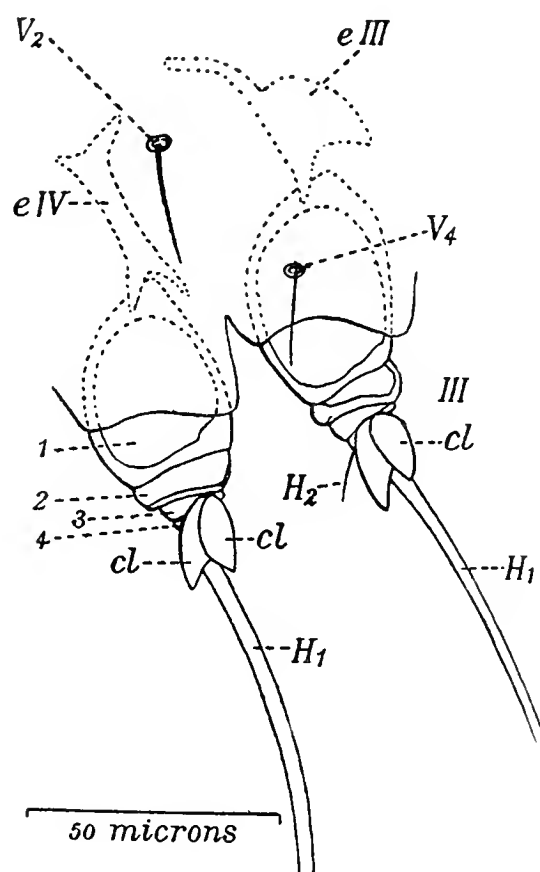


Fig. 12. *Sarcoptes scabiei* var. *equi*. Third and fourth legs of adult female seen ventrally. *cl*, claw; *e* III–IV, epimeres of third and fourth legs; *H* 1–2, setae of hind leg; *V* 2–4, ventral setae; 1–4, leg segments.

ventro-lateral aspect of the joint. It is about as long as the ambulacrum. The terms coxa, trochanter, femur, tibia and tarsus are used by many authors when referring to the five joints of the leg. This use is liable to the strong objection that the terms are technical names for certain parts of the insect leg, with which we cannot homologize the joints of the leg of the mites. As it is perfectly easy to speak of the "first joint" or the "fourth joint" I shall use this numerical terminology. Robin employs the terms hanche (rotule), exingual (trochanter), femoral, jambe, tarse, to designate the five joints.

The third and fourth pairs of legs (Fig. 12) resemble each other and differ from the first two pairs in the following respects; they are inserted as far from the middle line as the anterior are, but owing to the width of the body at this point they are completely concealed from above; they are smaller

than the anterior pair; they consist of four joints not five; they terminate in extremely long stiff bristles. If one examines either the fourth or the fifth leg one finds that the first joint is oval in shape, and covered for two-thirds of its length in a portion of the general integument of the body which is shaped like a cuff; the joint is about $2\frac{1}{2}$ times as long as the remaining three joints put together. On the cuff which covers the base of the third leg there is a short ventral seta (*V* 4). There is no corresponding seta on the cuff which covers the base of the fourth leg, though a seta in this position has been frequently figured. The second, third and fourth joints of either leg are successively smaller and smaller. The second and third joints are without setae; the fourth joint bears two claws, and the extremely long terminal seta (*H* 1) which corresponds to the ambulacrum of the anterior legs. This seta is equally long in the third and fourth pairs of legs. The terminal joint of the third pair of legs bears a minute seta (*H* 2) which is absent on the corresponding joint of the fourth pair of legs. (This seta is figured by Robin (1860) on both the third and fourth pairs of legs of *Sarcoptes scabiei* var. *hominis*.)

I can find no trace of a fifth joint though Robin (1860), who is an unusually careful investigator, describes the *five* joints of the posterior legs in detail. I believe he was misled by the fact that the second joint has a double outline, due to a transverse band of chitin which strengthens the extremity.

THE MALE.

The length of the male is 205 to 230 microns, the breadth about 170, and the ratio of length to breadth 100 to 75 or 85. The *dorsal surface* (Fig. 13) resembles that of the adult female except in the following particulars. The seta *D* 1 is shorter and stiffer than in the female; the plastron (*pl*) is relatively wider, and much longer, so that its length slightly exceeds its breadth, and the shagreening is coarser at the posterior end than at the anterior; only about 7 to 9 scales on each side are present. There is a pair of posterior plastrons (*ppl*) on the notogaster, between the dorsal and lateral rows of spines, and the shagreening of these is also relatively coarse. The shape of these can be seen from Fig. 13. There are only 12 spines (*sp*), arranged 3/3. The two pairs of anal setae (*A* 1 and 2) are present just as in the female: it must be due to an oversight that Munro (1919) figures one pair in his Fig. 3, two in his Fig. 6. The terminal seta *H* 1 of the third pair of legs is a conspicuous feature whether the mite is viewed from above or below, for it is as long as the body. The ambulacrum on the fourth leg is generally visible from above. There are three or four folds of integument on the side of the body on a level with the posterior plastron; they are very indefinite in number and degree of development.

The *ventral surface* (Fig. 14) differs very greatly from that of the female. The posterior end of the sternum or fused first epimeres extends considerably further back than a line joining the extremities of the second epimeres; these extremities are frequently described as forked, but it would be more true to

say that in shape they resemble a foot, with two sharp projections at the heel. The tocostome and its associated structures are of course absent. The ventral setae (*V* 1 to 4) and the lateral setae (*L* 1 and 2) have the same relative lengths as in the female, but *V* 3 is much more conspicuous, for instead of being concealed among integumentary ridges it is set on a flat place close to the epiandrium; the seta *L* 2 arises as in the female from a papilla, but its position is more distinctly ventral than in that sex. The third and fourth epimeres (*e* *III* and *e* *IV*) are united on each side to one another. The third is

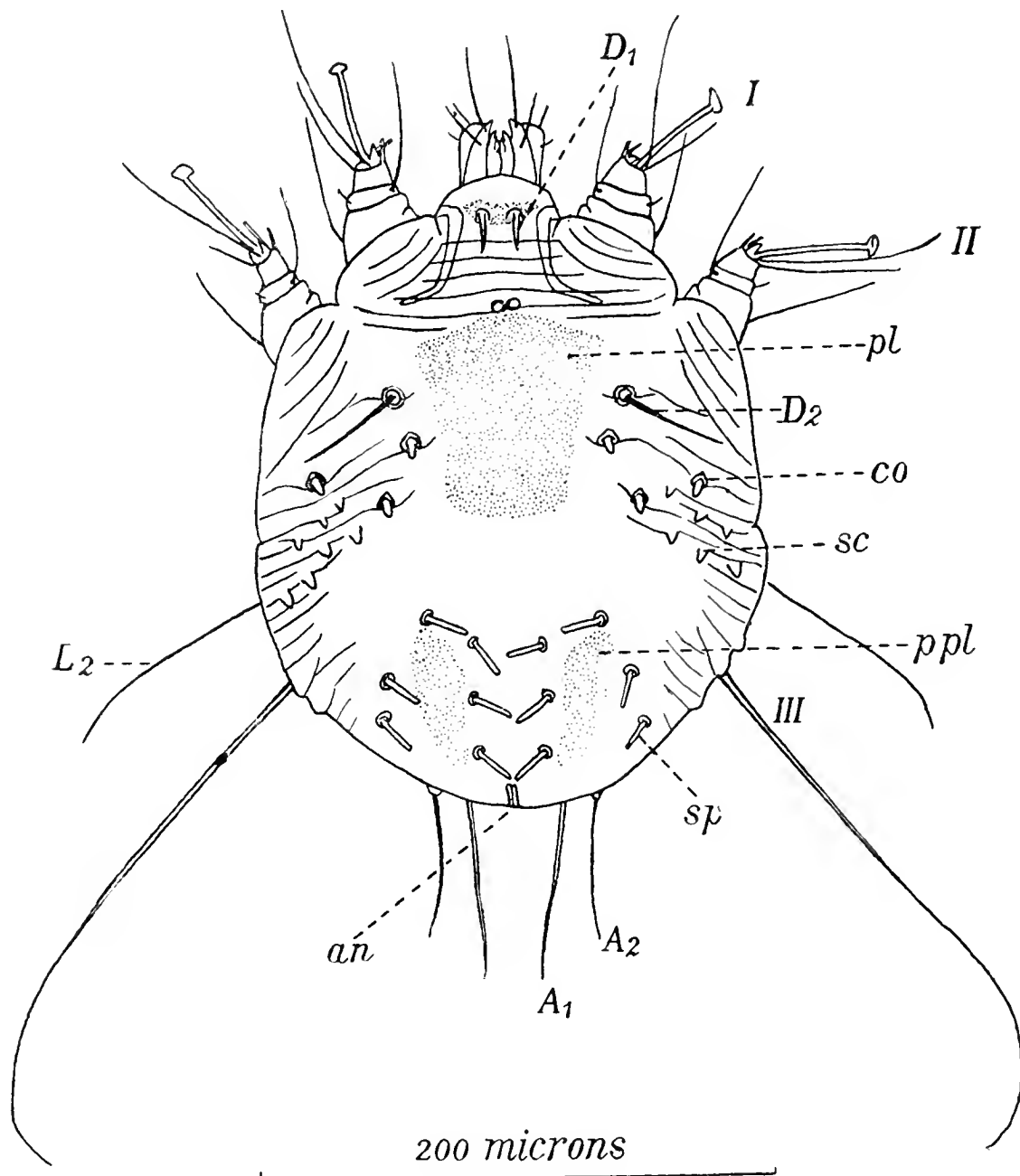


Fig. 13. *Sarcoptes scabiei* var. *equi*. Adult male, dorsal aspect. *A* 1-2, anal setae; *an*, anus; *co*, cone; *D* 1-2, dorsal setae; *L* 2, second lateral seta; *pl*, plastron; *ppl*, posterior plastron; *sc*, scale; *sp*, spine; *I*, *II* and *III*, legs (in the specimen figured the fourth leg is not visible from above).

very much bent, nearly transverse in position and provided with a wide flange; the fourth is straight and short. At the point at which they are united with one another, they have a definite surface by which they articulate with the epiandrium. In order to show this clearly I have very slightly separated these structures in Fig. 14. The epiandrium and the male organ is discussed separately below at some length. The *capitulum* of the male is similar to that of the female except that on the under surface the seta *C* 3 is slightly and *C* 4 considerably longer. In Fig. 13 the cheek-pieces have been displaced laterally

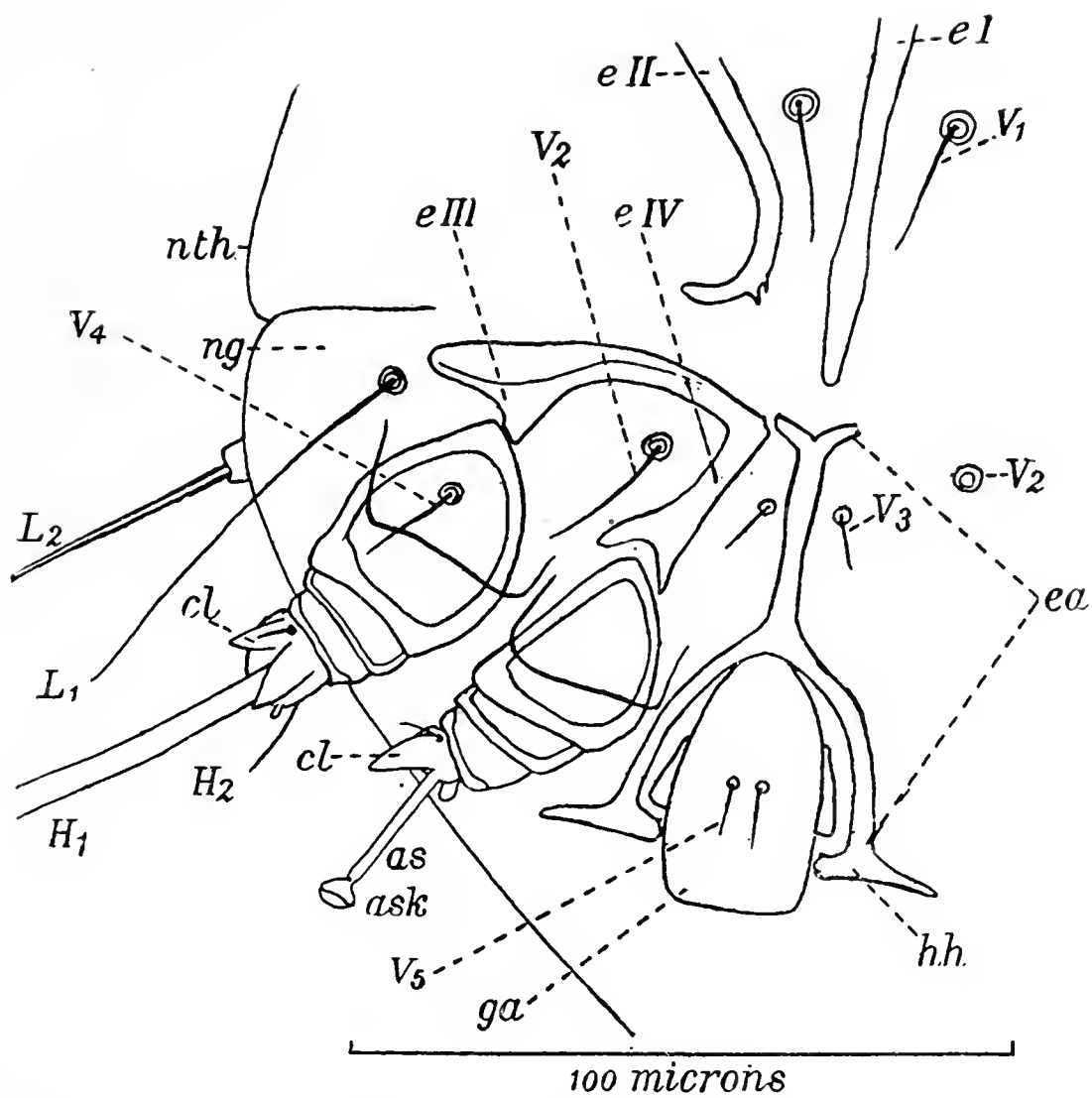


Fig. 14. *Sarcoptes scabiei* var. *equi*. Terminal part of ventral surface of adult male. *as*, *ask*, ambulacral stem and sucker; *cl*, claw; *e I-IV*, epimeres; *ea*, epiandrium; *ga*, genital apron; *H 1-2*, setae of hind legs; *hh*, hammer head; *L 1-2*, lateral setae; *ng*, notogaster; *nth*, notothorax; *V 1-5*, ventral setae.

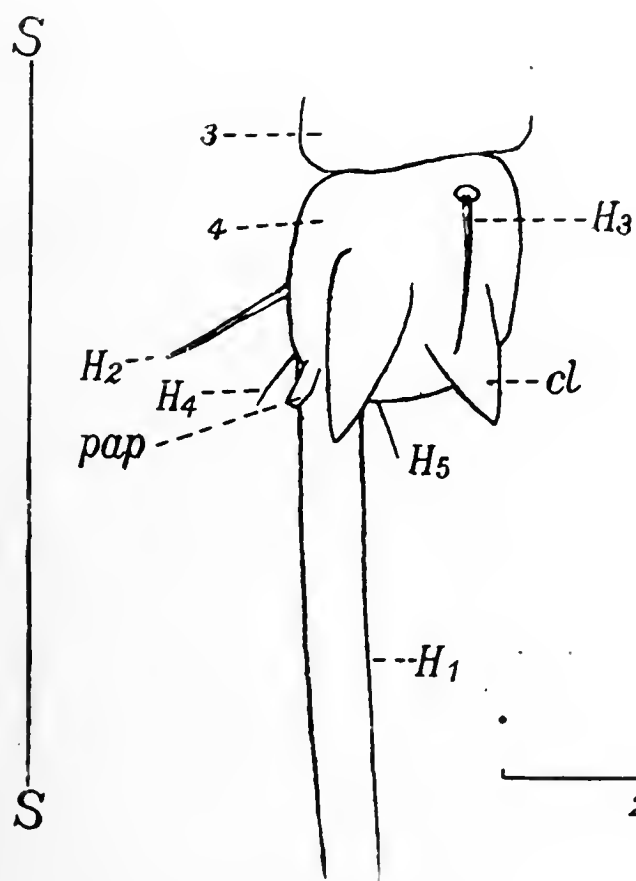


Fig. 15

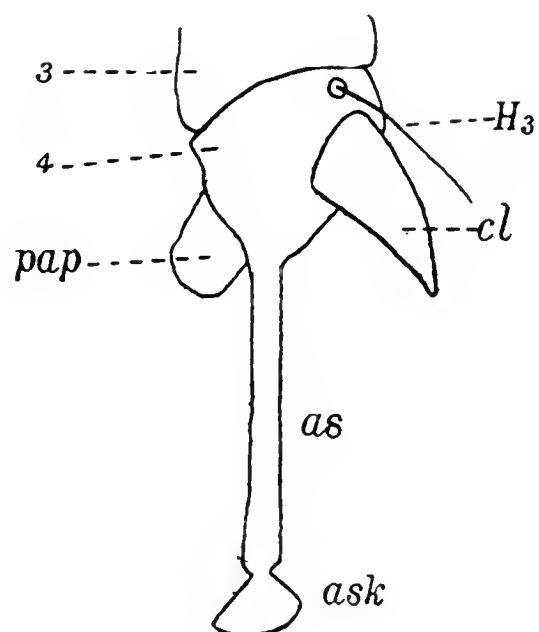


Fig. 16

Figs. 15 and 16. *Sarcoptes scabiei* var. *equi*. Terminal joints of third and fourth legs of adult male, seen ventrally. *cl*, claw; *H 1-5*, setae; *pap*, papilla; *SS*, sagittal plane; 3 and 4, third and fourth joints of leg.

and are therefore more noticeable than is usually the case; this is not a character of males in general. The first and second pair of *legs* resemble those of the female but the chitinous thickenings on the various joints are more strongly developed, so that the division of the leg into joints is more apparent. The chaetotaxy of the male and female anterior leg is identical except for the greater development of the minute seta *P* 4 in the male. As regards the spur, the claws and the ambulacrum, the male cannot be distinguished from the female. The third and fourth legs resemble those of the female in general structure as can be seen from Fig. 14, but differ in a number of small points. The seta *H* 1 of the third leg is extremely long, equal to the total length of head and body; *H* 2 is longer and stouter than in the female; in addition there are three setae, *H* 3, 4, and 5, which do not occur on the female; of these *H* 3 is a very small and fine seta, lying lateroventrally at the base of the lateral claw; *H* 4 and *H* 5 are short fine setae lying distal to *H* 2 and *H* 3 respectively. There is also present a minute papilla (*pap*) on the mesial side of the joint, distal to *H* 2. The fourth leg can at once be distinguished from that of all other stages by its possession of an ambulacrum in the place of the terminal seta; this ambulacrum is about half the size of the ambulacrum on the anterior legs. The setae *H* 2 and *H* 3 are present in the same relative positions as on the third leg; *H* 4 and *H* 5 are not present. The mesial claw is absent. In its place there is a large soft papilla.

Between the fourth epimeres of the two sides lies the complex genital apparatus. The most anterior part of this is the median rod of the epiandrium (*mea*), the "sternite" of Robin (1860). The anterior end of this rod is forked, and the limbs of the fork articulate on each side with a flat surface on the united third and fourth epimeres. In Fig. 14 the epimere and epiandrial rod have been separated by pressure in order to display the articular surfaces. The posterior end of the epiandrium consists of two curved epiandrial limbs (*eal*), springing from the median epiandrial rod. These limbs are the episternite of Robin; they diverge and pass backwards to terminate as two transversely placed hammer-heads (*hh*). The whole of this structure, the median rod, the limbs and the hammer head form the epiandrium (*ea*, Fig. 14), an organ which is comparable to an epimere; that is to say it is a rod-like internal thickening of the chitinous investment of the body, and its function is to supply a firm base for the erection of the genital organs themselves. From the concavity between the epiandrial limbs arises a flat lamella, the genital apron (*ga*), in shape something like a finger nail. This apron fills nearly the whole space between the epiandrial limbs, and exceeds that space posteriorly. It is an extremely thin, colourless sheet of chitin, bearing on its ventral surface a pair of small setae (*V* 5). Though it is quite transparent I have omitted from Figs. 14 and 17 the structures which lie beneath it, in order to simplify the figures. In nature the structures shown in Fig. 17 would be superimposed on these in Fig. 18. The parts which lie beneath the genital apron are shown in Fig. 18. In the concavity of the epiandrial limbs lies a horse-shoe shaped structure,

the hyposternite of Robin. It consists of three portions, the outer and inner rings, and the lateral rod. Of these the outer ring (*or*) is the largest, and at its posterior end is articulated with the hammer head. Part of the concavity

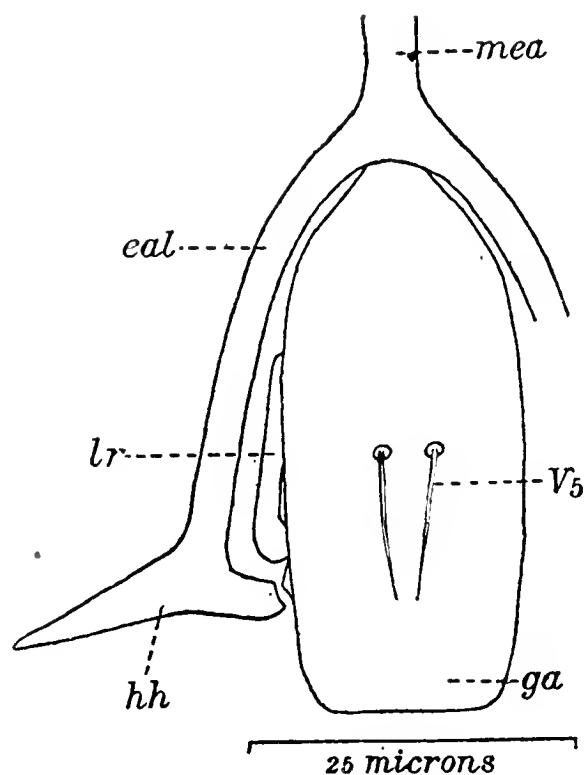


Fig. 17. *Sarcoptes scabiei* var. *equi*. Male genitalia seen from below. The structures behind the genital apron are not drawn, though actually the apron is quite transparent. *eal*, limbs of the epiandrium; *ga*, genital apron; *hh*, hammer head; *lr*, lateral rod of hyposternite; *mea*, medial epiandrial rod; *V 5*, fifth ventral seta, without counterpart in the female.

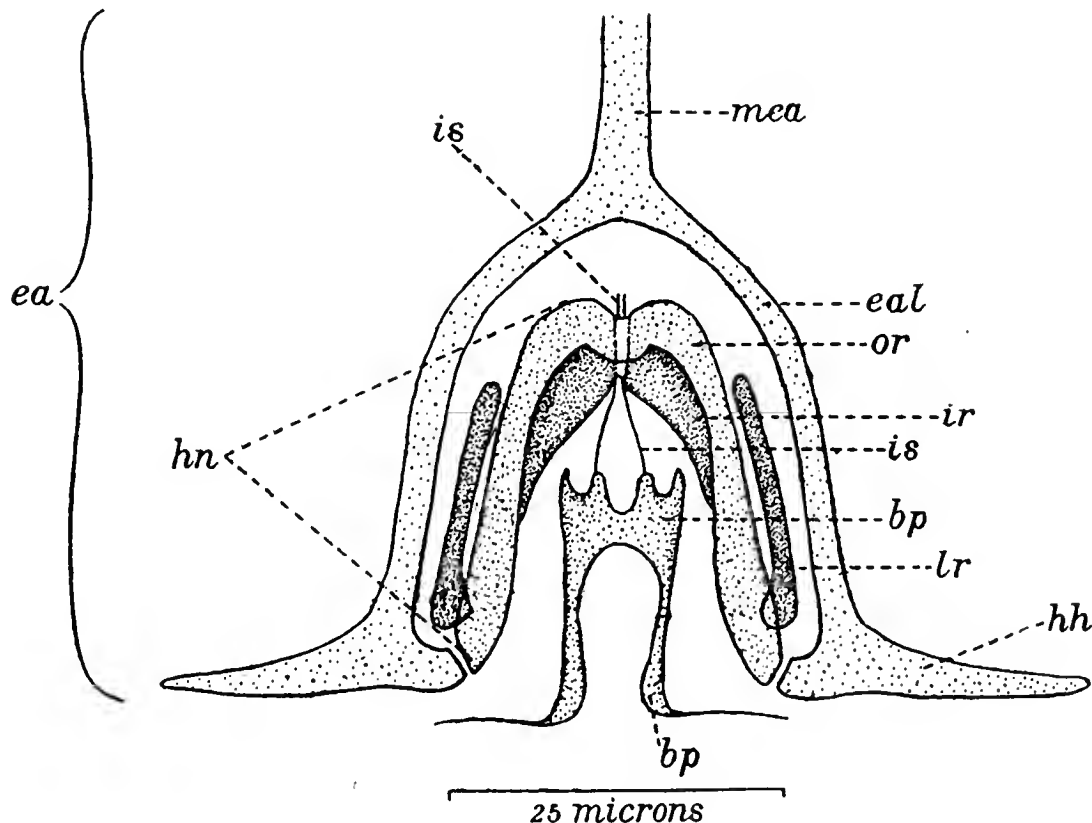


Fig. 18. *Sarcoptes scabiei* var. *equi*. Epiandrium as seen after removal of genital apron. *bp*, basis penis; *ea*, epiandrium; *eal*, its limbs; *hh*, hammer head; *hn*, hyposternite; *ir*, inner ring; *is*, intramittent spicules; *lr*, lateral rod; *mea*, median part of epiandrium; *or*, outer ring.

of the outer ring is filled by the inner ring (*ir*), which is intimately connected with the outer and lies on its dorsal surface. Through the most anterior part of both outer and inner ring passes a tubular passage (shown in dotted line

in Fig. 18); this passage opens in front at the bottom of a cup-shaped depression on the outer ring, and behind in the concavity of the inner ring. This passage contains the intromittent spicules. The third piece of the hyposternite, the lateral rod (*lr*), lies at the side of, and dorsal to, the outer ring. As I have said the intromittent spicules (*is*) pass through the passage in the inner and outer ring of the hyposternite, and their tips can be seen in the cup-shaped hollow at its end. They arise from a chitinous body, the "penis" of Robin; as I do not believe that this is an intromittent organ, I shall call it the basis penis (*bp*). It is shaped like a castle, with four projections like battlements upon its free anterior margin. It is from the two median projections that the intromittent spicules arise. At its posterior end the basis penis is wider and passes gradually into transverse folds of the integument.

The manner in which these very complicated genital structures are employed is not certainly known; so far as I am aware no one has ever observed *Sarcoptes* in copulation, and we must presume that the act is performed very rapidly; the closely allied *Psoroptes* is often seen walking about paired and the sexes do not separate when they are killed in alcohol. The current belief is that the female orifice for the reception of the male organ is within the anal opening; this is a fallacy (see p. 121). I agree with Gudden (1861) and Trouessert (1893) that copulation is performed through the minute opening on the copulatory papilla, and I believe that the duct which can be traced from this opening for a long way into the interior of the body leads to a spermatheca: I hope at a later date to deal with the internal anatomy of *Sarcoptes* and so settle this question; at present it is a matter of conjecture. The manner in which I believe the male organs are used is as follows. The whole of the epiandrium is a thickened part of the body wall; it therefore supplies a fixed base for the movement of the other parts. The hyposternite, hinged as it is to the hammer heads on each side can only move in one direction and is erected¹ so that it stands vertically to the surface of the body (or to the plane of the paper, in Fig. 16). The erection of the hyposternite would cause the erection of the basis penis, for they are locked together by the intromittent spicules; there is no difficulty in supposing this, for the basis penis is so attached to the body of the mite that clearly it can move in the sagittal plane of the mite, and in no other plane. When the hyposternite has been erected so as to stand perpendicularly to the ventral surface of the mite it appears probable that the copulatory papilla of the female is engaged in the cup-like hollow on the summit of the outer ring of the hyposternite. This brings the orifice on the papilla directly opposite to the tips of the intromittent spicules, which are I believe by this device enabled to find the extremely minute orifice. One has further to suppose, for I admit that it is a matter of supposition, that the vas deferens opens at some point on the summit of the basis penis, and that the spermatozoa

¹ I have failed to produce such erection by manipulating dead males, either freshly killed or macerated in water. Anyone who has attempted to manipulate the genital of a creature 225 microns long will understand why I failed.

pass along the intromittent spicules to the copulatory orifice and duct by capillarity. The weakness of this explanation is that it is from beginning to end a matter of hypothesis; its strength is that it explains the many peculiarities of the parts concerned, such peculiarities for instance as the minuteness of the spicules of the male, and of the papilla and duct of the female, and the presence of the cup-shaped hollow on the outer ring of the hyposternite.

THE IMMATURE FEMALE.

The length of the immature female is 230–250 microns, the breadth about 195, and the ratio of length to breadth about 100 to 80. It is therefore slightly larger than the male and a trifle wider on the average than male or female at any stage; as I have already stated there is a good deal of variation in these ratios, and I do not think that they have any practical value as a means of separating the stages. The line of division between the notothorax and notogaster is very indistinct or absent, in this and in the other immature stages. The *dorsal surface* (Fig. 19) resembles that of the adult female in nearly every respect. The dorsal epimere of the first leg is very weakly chitinized, and can only be found with difficulty; it is probably owing to its imperfect condition that an epaulette is not developed. The plastron (*pl*) is represented by a bare rectangular area, smooth and unfurrowed but differing from that of the adult in being not darker in colour than the surrounding integument and not shagreened or sculptured. The rudimentary stigma of Mégnin (*st*) is present. The dorsal armature is conspicuous because the individual cones (*co*), scales (*sc*) and spines (*sp*) are as large as those of the adult female. The scales stand in rather definite rows which are interrupted on the dorsum by the bare area (*ba*): this differs from the bare area of the adult in that though it is bare of scales it is crossed by integumentary ridges. The spines (*sp*) are in all respects similar to those of the adult, and the formula is 3/4; the last spine of the outer row is thinner than the others; possibly Munro overlooked it for he says that there are twelve spines in the immature female of *S. scabiei*. The dorsal lateral seta (*L* 2), and the two anal setae (*A* 1 and 2) are similar to those of the female.

The copulatory papilla and its duct are absent, an undoubted fact for I have examined a considerable number of immature females. This is very subversive of the accepted view that in the Sarcoptidae the immature female is the stage in which copulation takes place; the most likely explanation is that in the genus *Sarcoptes* it is the adult female alone which copulates. It is an exceedingly unfortunate fact that no one has as far as I know observed the act in this genus, and for the moment we must leave the matter in doubt. It is to be hoped that further light may soon be shed on this problem, which is one of the many interesting ones that remain to be solved.

The *ventral surface* is like that of the adult except in the following points: the genital operculum, tocostome and its setae are absent; the posterior end of the sternum is definitely forked, as is also the end of the epimere of the second leg. The *capitulum* and *legs* are in all respects like those of the adult

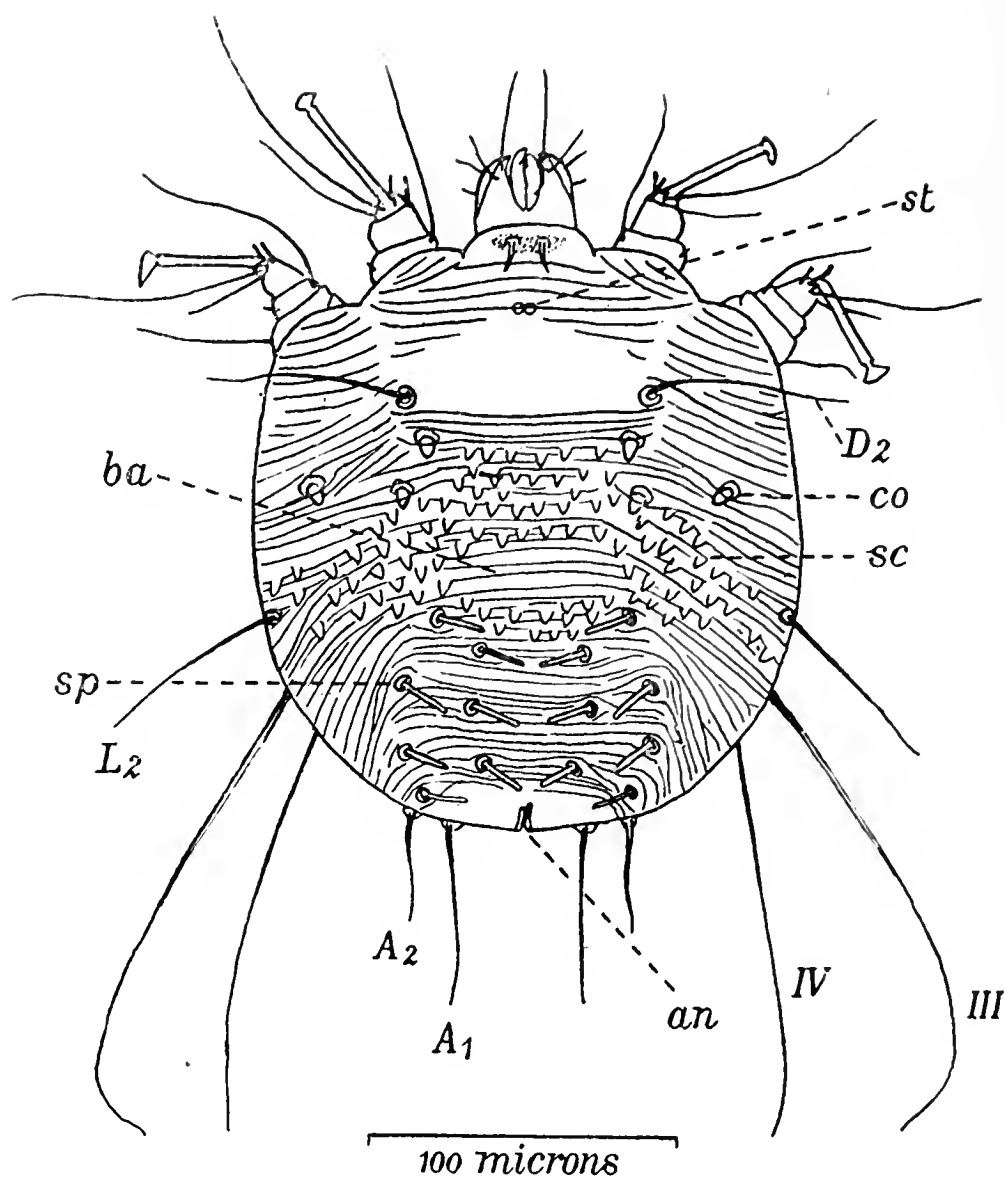


Fig. 19

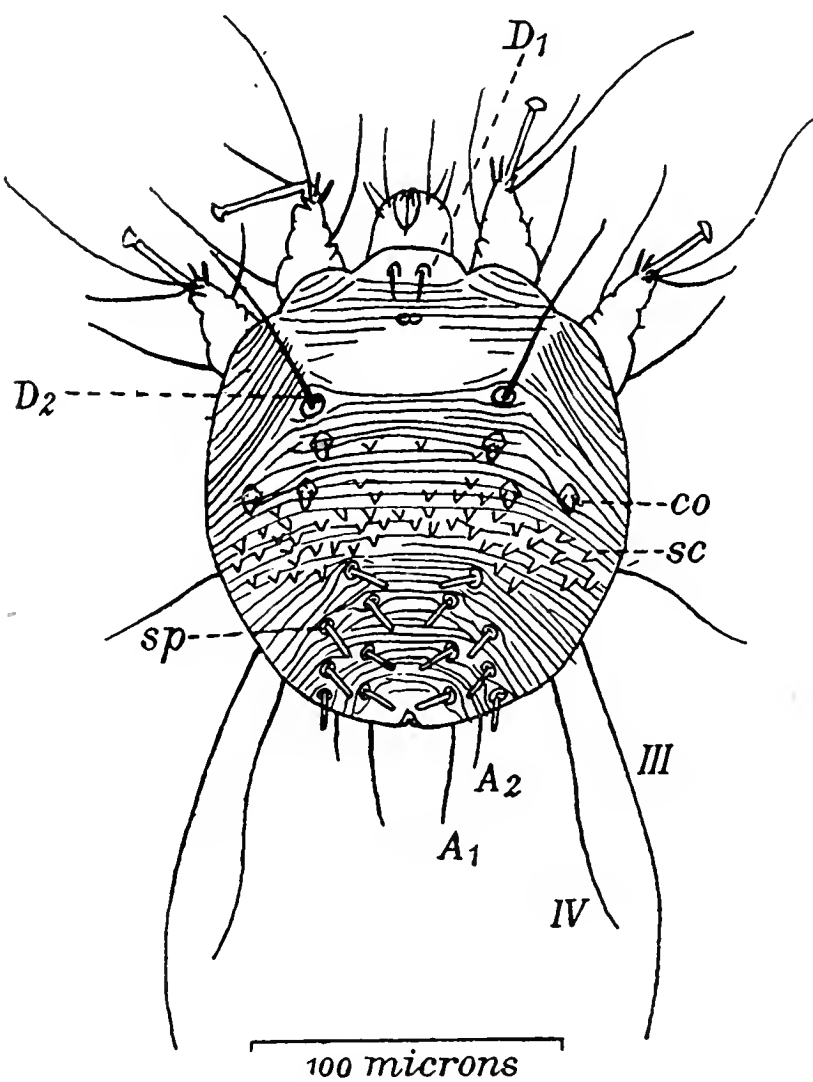


Fig. 20

Figs. 19 and 20. *Sarcoptes scabiei* var. *equi*. Dorsal views of immature female and of nymph respectively. *A* 1-2, anal setae; *an*, anus; *ba*, bare area; *co*, cone; *D* 1-2, dorsal setae; *L* 2, second lateral seta; *sc*, scale; *sp*, spine; *st*, probably rudiment of spiracle; *III* and *IV*, terminal setae (*H* 1) of third and fourth legs.

female; owing to their lower degree of chitinization it is not easy to distinguish the joints, and the skeletal rings and bands of thicker chitin; all these structures are, however, present as in the adult. The length of the long seta (*H* 1) of the third leg is about 80–82 per cent. of the body length.

THE NYMPH.

I am unable to find that the few nymphs I have seen fall into two groups, and can find no support for the statement that there are small nymphs which produce males and large ones which produce females. It is possibly the case but I have no evidence that it is so.

The nymph is about 175 microns long, by 135 broad, which gives a ratio of length to breadth, of 100 to 77.

The *dorsal surface* (Fig. 20) differs from that of the immature female in the following respects. The epistome and the seta *D* 1 are normal, but the small sculptured area from which *D* 1 springs in the adult and immature female is very indistinct in the nymph. The cones scales and spines are all smaller than in the adult and immature female. The scales, though smaller, are well formed and pointed, unlike those of the larva, and are just as numerous as in the immature female, but they do not extend across the dorsum posterior to the bare area, the posterior edge of which is therefore indefinite. The plastron is like that of the immature female, and differs from that of the adult female. The species are arranged as in the adult, the formula being $3/4$. *A* 1 and 2 are present just as they are in the adult female. (Munro states that there is only one anal seta in the nymph of *Sarcoptes scabiei* var *hominis*.) Only one lateral seta (*L*) is present. From its position it is probably *L* 1, but I am not certain of this.

The *ventral surface* resembles that of the immature female and larva in the forking of the posterior end of the sternum and second epimere, a point which is shown in Fig. 21 *B* of the ventral surface of the larva, and in the absence of the tocostome and its associated structures; it differs from that of the immature female in the absence of setae *V* 3 and 4.

The *capitulum* is identical with that of the immature and adult female in all respects. The *legs* differ from those of the immature female in the absence of the long seta *P* 1, and in the fact that *P* 7 and 8, the short stiff setae on the fifth joint, are represented by a single seta. In respect of the spur, the claws, and the details of the joints the legs are identical with those of the adult. The posterior legs are also quite normal. The terminal seta (*H* 1) of the third leg is equal in length to about 82 per cent. of the total length.

THE LARVA.

The length of the larva is 140–160 microns, the breadth about 100 microns and the ratio of length to breadth as 100 to 66.

The characters of the *dorsal surface* (Fig. 21 *A*) are as follows. The seta *D* 1 which springs from the base of the epistome is identical with that of the adult,

but the small sculptured area is absent. The plastron is as in the other immature stages, that is to say it is represented by a smooth rectangular space the surface of which is free from shagreening. Only two cones are present, the absent one being the anterolateral. The scales are few in number, small and blunt. The bare area is like that of the nymph, crossed by ridges, and only enclosed by scales in front and at the sides. Only five pairs of spines are present, and the formula is 2/3. As in the nymph one lateral seta only (*L*) is present.

The *ventral surface* (Fig. 21 *B*) shows the following features: the presence of *V* 1 and *V* 2 and absence of *V* 3 and *V* 4, as in the nymph; the forked end of the sternum and second epimere as in the nymph and immature female; the presence of a single anal seta (*A*) on each side and the absence of the fourth leg.

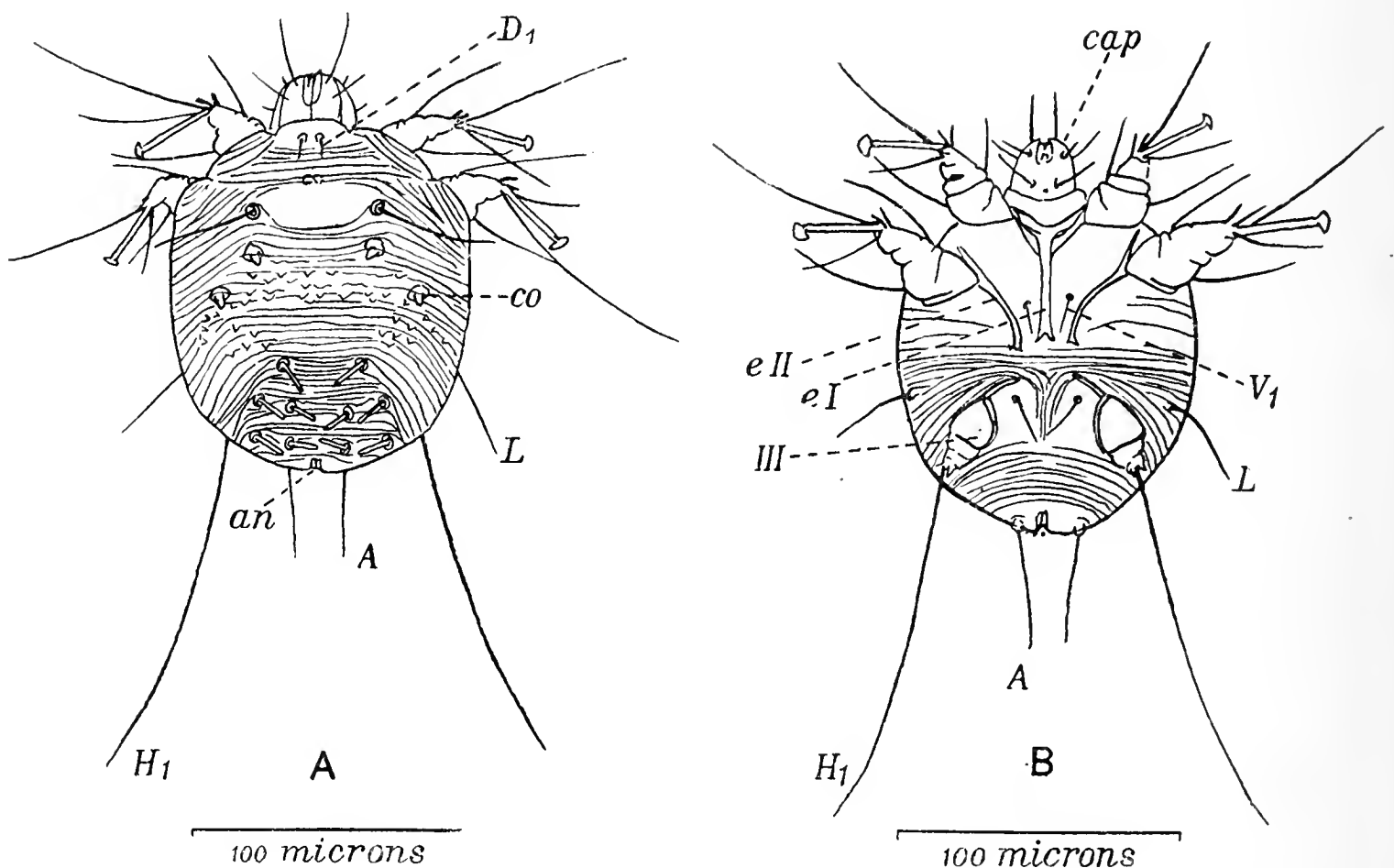


Fig. 21. *Sarcoptes scabiei* var. *equi*. *A*, dorsal, and *B*, ventral views of larva. *A*, anal seta; *an*, anus; *cap*, capitulum; *co*, cone; *D* 1, first dorsal seta; *e* I–II, epimeres; *H* 1, terminal seta of third leg; *L*, lateral seta; *V* 1, first ventral seta; *III*, third leg.

The *capitulum* is in all respects like that of the other stages. The *legs* are like those of the nymph, in the absence of *P* 1, and the presence of a single seta in place of *P* 7 and 8. In other respects they are normal. The fourth leg is absent. The ratio of the length of the seta *H*, of the third leg to the total body length is 89 per cent. to 97 per cent.

The gradual appearance of the adult characters through the larval, nymphal and immature female stages is perhaps worthy of being summarized. The capitulum and its setae appear to be identical in all stages, though I must confess that I have made no dissections of the capitulum of the early stages. The principal setae, *D* 1 and 2, *V* 1 and 2, *A* 1, and the general shape of the

leg, and of its several joints is the same in the larva as it is in the adult female. In the following respects the larva differs from the nymph; it lacks the fourth pair of legs, and the second anal bristle; its dorsal armature consists of only two cones and five spines, on each side, and of a relatively small number of scales. The nymph and larva lack the setae *V* 3 and 4, and *P* 1 and either *P* 7 or *P* 8, and one of the lateral setae (*L* 2?), but in other points resemble the immature female. The larva, nymph and immature female resemble each other but differ from the adult principally in the absence of the tocostome, and its setae and of the genital operculum; but also in the forking of the sternum and second epimere, the ridging of the bare area, the imperfect

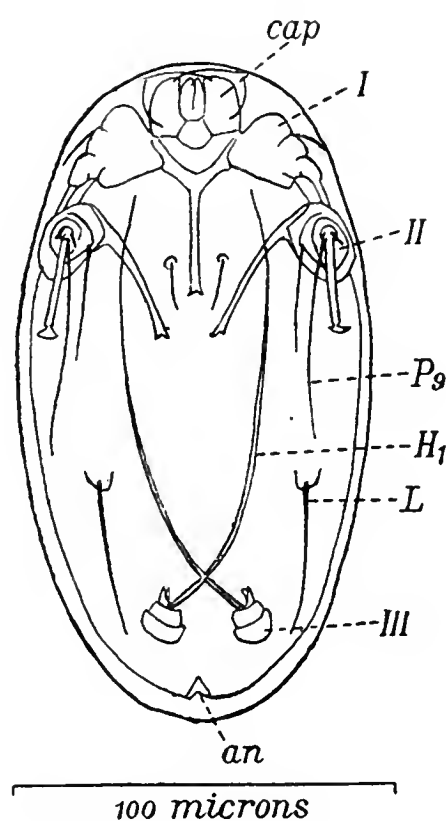


Fig. 22. *Sarcoptes scabiei* var. *equi*. Egg, in which is seen the ventral surface of a larva which is ready to emerge. Only some of the most conspicuous setae are shown. *an*, anus; *cap*, capitulum; *H* 1, terminal seta of posterior leg; *L*, lateral seta; *P* 9, ninth seta of second leg; *I*, *II*, *III*, legs.

separation of notothorax and notogaster, and the absence of shagreening on the plastron. It is therefore true to say that the assumption of the adult characters occurs gradually at each moult, and that no one moult more than another produces very marked changes.

THE EGG.

The length of the egg, measured without compression is 167–175 μ , the breadth 88–97 μ , and the ratio of length to breadth 100 to 51.5–56.8. The surface of the egg is smooth, without sculpturing. The example figured (Fig. 22) is about to hatch and the enclosed larva is seen by transparency.

KEY TO THE STAGES OF *Sarcoptes scabiei* var. *equi*.

The following key will probably be found useful; the egg stage is not mentioned.

A	{	Legs six. 150 μ long	Larva
		Legs eight	B
B	{	Fourth legs with ambulacra. Posterior epimeres united. Three plastrons.								
		Length 225 μ	Male
C	{	Fourth leg terminating in long seta ...								
		C
C	{	Toeostome and its setae present. Plastron shagreened. Length 390 μ								
		Adult female								
D	{	Toeostome absent. Plastron not shagreened ...								
		D
D	{	Bare area indefinite posteriorly. One lateral seta. Length 175 μ								
		Nymph								
D	{	Bare area closed behind. Two lateral setae. Length 240 μ								
		Immature female								

TECHNIQUE.

I have not found it necessary to develop a complicated technique. The following notes may be of service.

Collection. Scrapings should be made deeply to include the scurf and scabs next the skin. If the scabs are thick and only the superficial layer is collected it will be found to contain many larvae and very few of other stages. The scrapings may be folded up, as a doctor folds a medicinal powder, in a piece of paper. The mites migrate on to the paper in a very few hours; black paper therefore should be used so as to make them conspicuous. If the material cannot be examined fresh it may of course be preserved, or fixed, *en masse*.

Examination. It is essential to choose a fluid with the lowest possible refractive index, in order to render the sculpturing of the integument, and the fine setae transparent. Water is on the whole the most useful medium in which to examine specimens. Permanent mounts should be made in gum arabic (below). These mounts are as permanent as those made in balsam. The mite is placed in the gum alive, and dies rapidly in an extended position. Spirit specimens may be mounted in gum arabic, provided they are carefully washed in water. Levulose and lactophenol are also useful media, with lower refractive indices than gum arabic. They are unsatisfactory because they never harden, but at any rate they make useful semi-permanent specimens. Balsam is useless because it "clears" the specimen to such an extent that the chaetotaxy can only be made out with the utmost difficulty if at all.

The formulae of the mounting media are as follows. Gum arabic medium: distilled water 50 c.c., glycerin 20 c.c., gum arabic 40 gm., chloral hydrate 50 gm., cocain hydrochlor, 0.5 gm.; dissolve gum in water, add chloral hydrate and cocain, when dissolved add glycerin; filter if necessary. Levulose: saturated aqueous solution, evaporated to syrup on water bath, a trace of thymol added as preservative. Lactophenol: phenol crystals (puriss.) 1 gm., lactic acid 1 gm., glycerin 2 gm., water 1 c.c.

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ABBREVIATIONS.

The following abbreviations are used throughout the paper, in the plates and the figures:

A, anal seta of larva; *A 1*, *A 2*, anal setae of other stages; *an*, anus; *ap*, apodeme; *as*, ambulacral stem; *ask*, ambulacral sucker; *b 1-b 5*, chitinous bands of basis capituli; *ba*, bare area; *bd*, body; *bp*, basis penis; *bs*, base of chelicera; *C 1-C 4*, capitular setae; *cap*, capitulum; *cd*, condyle of chelicera; *ch*, chelicera; *ck*, cheek-piece; *cl*, claw; *co*, cone; *cp*, copulatory papilla; *ct*, copulatory tube; *D 1-D 2*, dorsal setae; *dg*, digit of chelicera; *e I-e IV*, ventral epimeres; *ea*, epiandrium; *eal*, epiandrial limbs; *el*, epaulette; *ep*, epistome; *er*, erect body on margin of lower lip; *ga*, genital apron; *gop*, genital operculum; *H 1-H 5*, setae of hind legs; *hh*, hammer head; *hn*, hyposternite; *ir*, inner ring of hyposternite; *is*, intromittent spicules; *k*, keel; *kn*, knob; *L*, lateral seta of larva; *L 1-L 2*, lateral setae of adult; *ll*, lower lip; *lr*, lateral rod of hyposternite; *mea*, median epiandrial rod; *ng*, notogaster; *nth*, notothorax; *or*, outer ring of hyposternite; *P 1-P 10*, pedal setae, on first two pairs of legs; *pap*, papilla on legs III and IV of male; *pl*, plastron; *pp*, palps; *ppl*, posterior plastron; *r*, integumentary ridges; *sc*, scales; *sp*, spine; *sr*, trochantal spur; *SS*, sagittal plane; *st*, ring-shaped organs, possibly rudiments of stigmata; *t*, tongue, or languette; *tb*, tubercle of digit of chelicera; *tc*, tocostome; *ts*, tocostomal seta; *V 1-V 5*, ventral setae; *z*, the "menton" of Robin; *I*, *II*, *III*, *IV*, the first, second, third and fourth legs; 1-5, the joints of palp or leg numbered from base.

ON THE *SARCOPTES* OF MAN¹.

BY P. A. BUXTON, M.A.,

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(With 9 Text-figures.)

OUR knowledge of the systematics of the genus *Sarcoptes* is in an unsatisfactory state. About a score of species have been admitted, many of which have never been seen since they were described. Many of the descriptions date from between sixty and seventy years ago, a period when microscopes and microscopical technique were barely adequate to cope with so difficult a genus as *Sarcoptes* is. In order to provide a basis for further study of *Sarcoptes*, Warburton (1920) published a critical survey of our knowledge regarding these mites. His review of the literature has proved invaluable to myself, and is the foundation on which I have worked. In the foregoing paper (pp. 114–145) I published an only too lengthy description of the *Sarcoptes* of the horse, finding that a full description of one form was necessary as a basis for comparison. Since we do not know what anatomical points are of systematic importance, attention was given to all details of the external anatomy.

I propose to deal now with the *Sarcoptes* of man. It is commonly held that the “itch” is caused by a species or variety peculiar to the human race (*Sarcoptes scabiei* var. *hominis* (Hering)) and that this mite is separable on anatomical grounds from the sarcopts of other animals; it is believed that man is also attacked by “*S. scabiei-crustosae*” Fürst., and that this mite, which produces symptoms graver than those of itch, is a “good species,” separable from *S. scabiei* and from the species attacking quadrupeds by definite anatomical characters. This second sarcopt is however very little known, and the “Norwegian Itch” or “Crusted Scabies,” of which it is the cause, is a rare disease.

1. *Sarcoptes scabiei* var. *hominis* (Hering).

In comparing the common *Sarcoptes scabiei* of man with that of the horse particular attention has been devoted to the characters on which previous authors separated their species and the following conclusions have been reached. Canestrini and Kramer give 300–450 by 250–350 microns as the dimensions

¹ Investigation carried out with the aid of a grant from the Ministry of Agriculture and Fisheries.

of female *S. scabiei*, and 450–500 by 310–370 microns as those of the sarcopt of the horse. Gerlach separated his "*S. equi*" from *S. scabiei* on its greater size and greater relative length in the ovigerous female. I have shown (*loc. cit.*) that variation in size and proportions occurs even when mites are measured living under standard conditions. The relation between width and breadth is altered by pregnancy. My material of the common *S. scabiei* was collected by Mr J. E. M. Mellor during the war from a large number of different cases both civil and military, therefore the possibility is excluded that we are dealing with a case of equine scabies in man. I have been unable to obtain sufficient living material from man to give the measurements of a series of specimens, but I do not believe that differences of size are of the least use in separating species and varieties of *Sarcoptes*. The plastron in adult females from man is frequently, but by no means always, less highly pigmented than it is in those from the horse. There is no difference to be detected in the three plastra of the male. The bare areas (rugose areas) vary in their development among

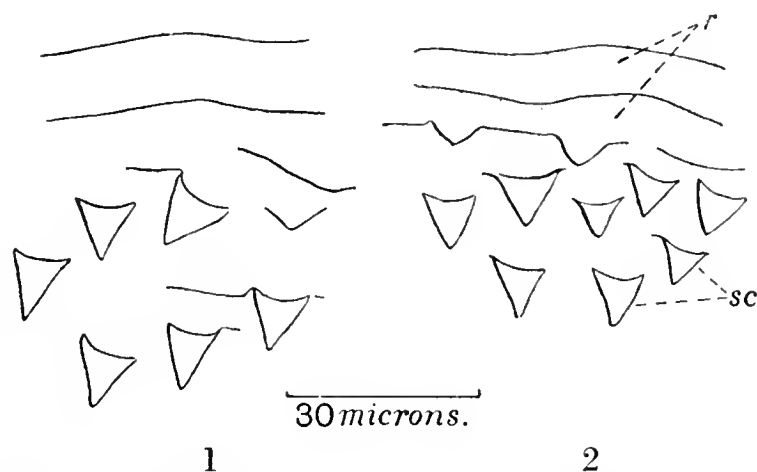
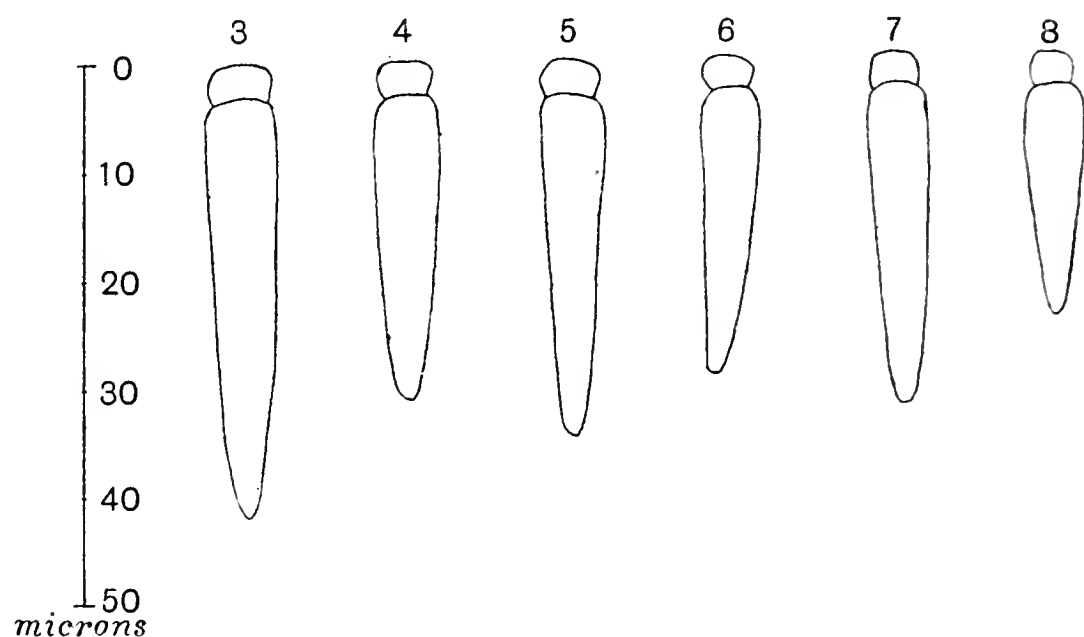


Fig. 1. *Sarcoptes scabiei* var. *hominis*, adult ♀. Scales from left side immediately behind the antero-median cone.

Fig. 2. *S. scabiei* var. *equi*, adult ♀. Scales from same area. The two figures drawn to the same scale with a Zeiss camera lucida. *r*, integumentary ridges; *sc*, scales.

specimens from man just as they do among specimens from the horse. In ovigerous females from either host either none or one or two may be present, or a large one partly divided by a row or two of scales; if two are present one or other may be very small. Neither the plastron nor the bare areas are of any systematic importance. The scales in specimens from man are said to be pointed and longer than broad; in those from the horse, small and scarcely longer than broad (Canestrini and Kramer). Hirst (1920), speaking of specimens from man, refers to the "dorsal scales being distinctly longer as compared with their breadth than in *Sarcoptes scabiei* var. *equi*." I find that the differences are so slight, and subject to such individual variation, that they are useless for purposes of discrimination. Scales vary greatly in size on any one mite, but the largest on ovigerous females from the horse do not exceed 8.5 microns in length. In specimens from man they reach 10–11 microns, as a maximum, but on many mites no scale longer than 9.5 can be found. Figs. 1 and 2 show scales from the same part of the dorsum of females from the horse

and from man. In shape I can detect no differences provided that a sufficient number of scales be examined. Scales are slightly concave or slightly convex at the side, or concave on one side, convex on the other; these variations occur quite irrespectively of the host from which the specimen was derived. Canestrini and Kramer state that the spines of *S. scabiei* var. *hominis* are long and pointed, those of var. *equi* blunt. This distinction is borne out by measurements, and I have measured the second spine of the inner row, and the second spine of the outer row of a series of ovigerous females. That of the inner row measures 31–33 microns in *Sarcoptes* from the horse, that of the outer row 25–28 microns: corresponding figures for *S. scabiei* var. *hominis* are 35 microns and 29–30 microns. There is a further small and rather inconstant difference in the greater stoutness of many of the spines of the equine



Figs. 3 and 4. *Sarcoptes scabiei* var. *scabiei-crustosae* (Fürst.), adult ♀. Second spine of inner (3) and outer (4) rows.

Figs. 5 and 6. *Sarcoptes scabiei* var. *hominis*, adult ♀. Second spine of inner (5) and outer (6) rows.

Figs. 7 and 8. *Sarcoptes scabiei* var. *equi*, adult ♀. Second spine of inner (7) and outer (8) rows. All the figures are drawn to the same scale with a Zeiss camera lucida.

Sarcoptes. The slight curve in Fig. 6 is due to the spine being viewed laterally; all spines are slightly curved, and the curvature cannot be used for separating races. I can detect no other differences whatever between *S. scabiei* var. *hominis* and var. *equi*. I have examined with particular care the cones, the trochanteric claw, and the chaetotaxy of the capitulum, body and legs; taxonomic differences have been described in all these structures, but I can find no differences whatever. I have examined adult males and females, but not the immature stages.

I conclude that the *Sarcoptes* of the horse and the common species found on man cannot invariably be separated. Certain minute differences exist in scales and spines but they are not constant, and the measurements overlap. Other characters which have been used for separating these forms appear to be wholly useless. It is convenient to regard these forms as varieties, as many

previous writers have done; this is more justifiable on physiological than on morphological grounds. The common itch mite of man stands as *Sarcoptes scabiei* de Geer, 1778, var. *hominis* (Hering, 1880); that of the horse as *S. scabiei* var. *equi* (Gerlach, 1857).

2. "*Sarcoptes scabiei crustosae*" Fürstenberg.

Fürstenberg differentiated a species under this name, giving as its character its shape, which is "rounded, slightly longer than broad," and the greater length of one of the lateral setae, almost certainly the seta which I have lettered *L* 2. Canestrini and Kramer say "dorsal scales blunt; no bare area (Blösse). Spines long, acute, slightly bent. Anterior arm of epiandrium strongly developed and reaching the epimere. Male 170×150 microns, female 410×340 ."

I have examined a series of specimens obtained by Dr Wallace Beatty from a case of Norwegian crusted scabies, and kindly lent to me by Col. A. Alcock, F.R.S., I.M.S. There is no doubt that these specimens came from genuine cases of Scabies Norvegica. Beatty has figured two cases (1913, 1915) and I have examined mites from each of these. I find that adults may be separated from those of typical *S. scabiei* var. *hominis* on one character only, and that is the greater size of the spines (Figs. 3 and 4). One must always exclude the fourth spine of the outer row from one's measurements because of its peculiar shape and proportions: the other spines measure 34 microns or more, and are also slightly thicker than those of *S. scabiei* var. *hominis*. The second spine of the inner row measures 42 and 45 microns in two females examined, the second in the outer row 36 and 42 microns. I can find no other points of difference whatever. The specimens at my disposal have been dried, and it is therefore impossible to measure length and breadth, but their size and proportion do not appear to differ from those of typical *S. scabiei*. As the only characters by which this mite may be distinguished require an oil-immersion lens and a micrometer for their proper appreciation it appears best to relegate it to varietal rank. It is unfortunate that this form was originally named *Sarcoptes scabiei crustosae*. The third name was not used in a varietal or subspecific sense, and there is no doubt that "*scabiei crustosae*" was regarded as a specific name. It is technically invalid, because it does not conform to the binomial principle. Later writers have regularized matters by writing *scabiei-crustosae* with a hyphen, as an emendation. The name of this variety is therefore *Sarcoptes scabiei* var. *scabiei-crustosae* (Fürst.).

3. Norwegian crusted scabies.

Dr Wallace Beatty's specimens are interesting in another way: the preparations, which are made in Farrant's medium, are full of a species of *Aspergillus*. That the mould is not a contamination is proved by the fact that the conidiophores of the mould are to be found in all parts of the preparation. If

the mould had invaded the preparation after it was made conidiophores would be found only at the edge of the cover glass. I can find no previous reference to the occurrence of *Aspergillus* in the crusts formed in this disease, and one or two leading dermatologists whom I have consulted inform me that they are not aware that it has been previously recorded. We may be dealing with an accidental infection of the crust by a common saprophytic mould. In support of this Dr Aldo Castellani informs me that *Aspergillus* is extremely common in old scrapings of a number of skin diseases. On account of this it was believed for many years that the causative agent of *Tinea imbricata* was an *Aspergillus*. We must not dismiss altogether the possibility that Norwegian crusted scabies is a condition produced by a secondary infection of a case of ordinary itch with *Aspergillus*. Writers of most modern text-books of dermatology (Hyde and Montgomery, Hartzell, Sutton, Sequeira), regard the crusted variety as common scabies occurring in a dirty and negligent individual. I am inclined to agree with them and to explain the minute differences between the common *Sarcoptes scabiei* and *S. scabiei* var. *scabiei-crustosae* as adaptations of the variety to its environment. Incrustations, similar to those of crusted scabies in man, are common on mangy horses; but I am unable to find any difference between mites from a horse which had been covered with crusts for many weeks, and mites from milder cases.

The view is also held that *S. scabiei* var. *hominis* and *S. scabiei* var. *scabiei-crustosae* are distinct organisms producing essentially different diseases: this was the view held by Fürstenberg and by Canestrini and Kramer. Against this view one may urge the minuteness of the morphological differences between the species and the variety. Beatty's second case (1915) was believed to have been infected from a case of common scabies. Moreover Norwegian crusted scabies is such a rare condition that were its causative mite really distinct from *S. scabiei* var. *hominis* it would long ago have become extinct. Norwegian crusted scabies is a rare disease. It is much to be desired that some one who is fortunate enough to see a case should infect a few volunteers in order to discover whether ordinary itch or the crusted variety is induced, and whether, after one or two generations, the mites can in any way be distinguished from typical *S. scabiei* var. *hominis*. A search should also be made for moulds.

A third view has been held, that Norwegian crusted scabies is due to an infection of man with a *Sarcoptes* derived from some other animal: Mégnin attributed it to *S. lupi*. The *Sarcoptes* of several of the common domestic animals (notably *S. scabiei* var. *equi*) produces in man a disease less, and not more, severe than ordinary human scabies.

An anomaly in chaetotaxy.

Fig. 9 shows an abnormality in the setae of an ovigerous female of *Sarcoptes scabiei* var. *hominis* collected by J. E. M. Mellor from our laboratory assistant Mr Reid after he had been artificially infected from a military case of scabies. The anomaly occurs on the left side only and consists in the reduplication of

seta *D* 2. The normal *D* 2 is present on both sides, and in its normal position: lateral to it on the left side is a seta one half of its length, and much finer. This anomaly is perhaps worth recording as the great setae are believed to be of considerable systematic importance.

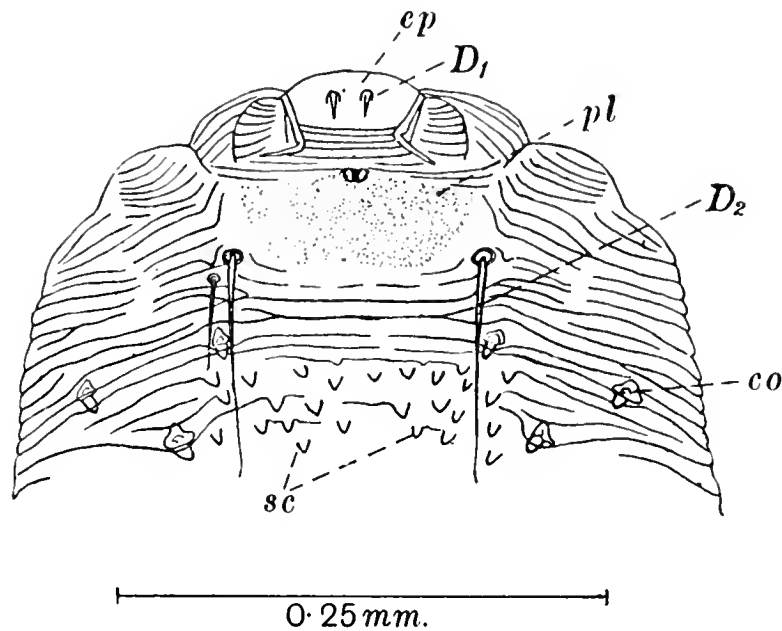


Fig. 9. *Sarcoptes scabiei* var. *hominis*, adult ♀. Part of dorsal surface showing reduplication of seta *D* 2 on left side: capitulum and legs omitted. *co* cone, *D* 1 and *D* 2 dorsal setae, *ep* epistome, *pl* plastron, *sc* scales.

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NOTES ON *RICKETTSIA*¹.BY EDWARD HINDLE²,*Professor of Biology, School of Medicine, Cairo.**(From the Quick Laboratory, Cambridge, and the Biological Dept.,
School of Medicine, Cairo.)*

INTRODUCTION.

AMONG the recent advances in our knowledge of the aetiology of human diseases, one of the most interesting is the discovery of a new group of somewhat problematic organisms which have been grouped together under the generic name *Rickettsia*. Up to now their presence has been shown, more or less satisfactorily, to be associated with three types of human fevers, viz. typhus, trench fever and possibly Rocky Mountain spotted fever, and in the last few years a certain number of papers have appeared on this subject, the most comprehensive of which are those published by the British War Office Committee and the American Commission on trench fever. In addition it has been suggested that many other human diseases are caused by these organisms, but the evidence is so very unsatisfactory that it may be disregarded.

Up to the present the papers which have been published on *Rickettsia* are concerned mainly with experimental and clinical investigations. With the object, therefore, of attempting to throw some light on the nature of the organisms themselves, in spring, 1919, at the suggestion of Professor Nuttall, I took up the study of the *Rickettsia* bodies associated with trench fever.

The investigation eventually resolved itself into three main divisions. First, the examination of a series of lice from persons known not to have been exposed to trench fever or typhus, in order to determine whether or not they contained *Rickettsia*; secondly, observations on *R. quintana* obtained by feeding lice, known to be uninfected, on a patient suffering from trench fever; finally, the examination of a number of blood-sucking insects in order to see how widespread is the occurrence of infection with *Rickettsia*.

(1) EXAMINATION OF UNINFECTED LICE.

One of the arguments which has been used against the view that *Rickettsia* are parasites is the statement that these bodies may be found in uninfected lice and are normal constituents of the alimentary canal. Thus Brumpt (1918),

¹ Read before the Kasr-El-Ainy Staff Club, School of Medicine, Cairo, on 13 Nov. 1920.

² The expenses of this investigation have been partly defrayed by the aid of a grant from the Medical Research Committee.

as the result of an examination of lice collected from prisoners of war at Rennes, found *Rickettsia* in 53 out of 72 body-lice, whilst 27 head-lice were examined with negative results. Da Rocha Lima (1917) found similar organisms in lice collected from persons in Volhynia, but he remarks that they only occurred very sparingly and were confined to the lumen of the alimentary canal, only rarely invading the epithelial cells. To this organism he gave the name *Rickettsia pediculi*, and used the latter character to distinguish it from *R. prowazeki*, in which invasion of the epithelial cells is the usual occurrence. Various other Continental observers have also recorded the finding of *Rickettsia* in lice collected from persons who had not suffered from either typhus or trench fever. On the other hand Arkwright, Bacot and Duncan (1919), as the result of a careful examination of 245 specimens reared in the laboratory, found only one louse infected with *Rickettsia*.

Through the kindness of Mr Bacot I obtained a number of lice from the same stock that had been used in their experiments. These lice for many generations, extending back for more than three years, had been fed on a healthy person and had never produced any infection.

At the Quick Laboratory these lice were fed twice daily in the manner described by Nuttall (1917), on a laboratory attendant who had never been exposed to the possibility of infection with either typhus or trench fever.

When not in use the lice were kept in an incubator at 30° C. As a louse completes its life cycle in a period of less than a month, the stock used had been fed on healthy persons for at least forty generations. The faeces from these lice were examined at frequent intervals during a period of four months, but on only two occasions were any *Rickettsia* bodies found present. In addition the gut contents of 420 lice were examined by making smears, and staining with Giemsa in the usual way.

In the case of two individuals, bodies resembling *Rickettsia* were found, but the examination of the remainder was uniformly negative.

In addition 112 lice from various other sources were examined without finding any of these bodies.

The results of this examination of uninfected lice are in complete agreement with those obtained by Arkwright, Bacot and Duncan (1919), and by Wolbach, Todd and Palfrey (1920) in Poland. Only two naturally infected lice were found in 532, and therefore it is evident that this strain of lice is practically free from *Rickettsia* infection. The positive results obtained by Continental observers may be explained on two hypotheses.

(1) That owing to the prevalence of typhus and trench fever on the Continent during the war, the lice had become infected with *Rickettsia* by feeding on patients suffering from these diseases; and (2) that there is a species of *Rickettsia* which lives in the alimentary canal of the louse and is non-pathogenic to man.

Although the above-mentioned results would seem to favour the first hypothesis, it is difficult to explain all the positive results on the supposition

that the lice had fed on infected patients. In the first place Arkwright, Bacot and Duncan (1919), and the present writer found *Rickettsia* in three cases, and unquestionably these lice had never fed on any one infected with either trench fever or typhus. Moreover Töpfer (1916) and Da Rocha Lima (1917) were both well aware of the possibility of their lice having been contaminated and still came to the conclusion that there is a distinct parasite, *Rickettsia pediculi*, occurring in the human louse. The results of the examinations of other species of lice also confirm the view that there may be a *Rickettsia* specific to the body-louse.

(2) OBSERVATIONS ON *RICKETTSIA QUINTANA*.

Through the courtesy of Major-General Sir David Bruce, Chairman of the Trench Warfare Committee, a case of trench fever at the Mill End Hospital, Hampstead, was placed at my disposal. Unfortunately, this patient had only just recovered from an attack of roseola, but as his was the only case available, and he was suffering from an unmistakable attack of trench fever, it was decided to use him for obtaining *Rickettsia quintana*. Major Byam very kindly arranged for two boxes of lice from the Quick Laboratory to be fed daily on this patient for a period of 14 days. These lice were then examined by various methods and 14 out of 22 were found to be infected with *Rickettsia* in large numbers.

The faeces from both boxes, which previously had shown no sign of these organisms, were also examined and found to be infected. Although the amount of material was small, the appearance of the films made from infected lice was so strikingly different from any of the films made from uninfected lice that the presence of the *Rickettsia* was unmistakable. When seen singly or in very few numbers, it is possible to mistake them for staining artefacts, etc., but in the cases mentioned their definite shape and occurrence in clumps gave them a very characteristic appearance. However, as dried films stained with Giemsa are liable to produce artefacts, especially in the case of gut contents, some of the lice were fixed in various ways and afterwards sectioned and the sections stained.

The most satisfactory results were obtained by fixing in sublimate alcohol containing 5 per cent. acetic acid and afterwards staining by Giemsa's wet method, using acetone instead of alcohol for dehydrating. Other lice were fixed in Flemming's solution, Bouin and Gilson, respectively, and afterwards stained by different methods.

The aniline stains gave but indifferent results, as *Rickettsia* seem to have very little affinity for the ordinary staining reagents. When stained with iron haematoxylin it was very difficult to obtain any degree of differentiation that would clearly distinguish them from surrounding structures. When the sections were under-differentiated it was possible to recognise the outlines of the *Rickettsia*, but the organisms always appeared slightly smaller than in sections stained with Giemsa.

In its staining reactions *Rickettsia* shows a very close resemblance to *Spirochaetes*, as the cytoplasm seems to contain a relatively small amount of chromatinic substance which is diffused evenly through it. For demonstration purposes Giemsa or Leishman is by far the best stain to employ, as the colouring matter is precipitated round the surface of the organism, and therefore it appears somewhat larger and has a fairly distinct outline.

As noticed by previous observers, *Rickettsia* from the gut of the louse presents the appearance of round, oval, or diploid bodies, often occurring in clumps which are obviously the result of continued division without separation of the individuals. The parasite stains a purplish-blue colour with Giemsa and its dimensions may be estimated as about 0.3μ in diameter by $0.3-0.5\mu$ in length. When dividing, the chromatinic material is concentrated at the opposite poles of the cell, thus giving the appearance of two darker stained bodies united by a somewhat lightly stained substance. Eventually the two halves separate, but as division seems to proceed rapidly these so-called diploid forms are usually present and form a characteristic feature.

The examination of sections and wet films has not resulted in finding any structure in *Rickettsia* which cannot be observed just as well in dried films, and as the latter are much simpler to prepare their use is recommended for this organism.

With regard to the occurrence of *R. quintana* in the alimentary canal of the louse the parasites are confined to the lumen of the gut and I have not observed their entry into the epithelial cells lining the alimentary canal or into any other parts of the body. The organisms seem to become attached to the surface of the epithelial cells of the midgut, and as they multiply gradually fill the lumen of the gut and extend backwards down the intestine, and eventually are voided together with the faeces.

The results of these observations confirm those obtained by Arkwright, Bacot and Duncan (1919) in their important series of experiments on the association of *Rickettsia* with trench fever. With regard to the systematic position of the organisms themselves, there is nothing to distinguish them from bacteria except their minute size.

The multiplication in the mid-gut and subsequent extension into other parts of the alimentary canal resembles that of *Bacillus pestis* in the gut of the rat flea. The insect host, in the case of *R. quintana*, merely furnishes a living culture tube in which the organisms grow and multiply without passing through any cyclical changes comparable with those that take place in the case of protozoa. The negative period of four days which intervenes between the ingestion of blood containing *Rickettsia* and the faeces of the louse becoming infective, is probably merely the result of the *Rickettsia* not being present in sufficient numbers to produce any infection, for it is well known in the case of other diseases that the inoculation of less than a minimum number of organisms does not produce any noticeable infection.

(3) OBSERVATIONS ON OTHER SPECIES OF *RICKETTSIA*.

Being unable to obtain additional cases of trench fever owing to its gradual disappearance after the war, a number of insects from various sources were examined in order to see whether other species of *Rickettsia* could be found.

Rickettsia melophagi.

Through the kindness of Messrs Cooper, Sons and Nephew, Watford, large numbers of *Melophagus ovinus* were obtained from sheep in various parts of the British Isles. The examination of these sheep-keds revealed the presence of *Rickettsia* in the alimentary canal of a large proportion of these insects. It seems to be fairly widespread for in addition to Germany, where it was noticed by Nöller (1917) and Sikora (1918), I have found it in *Melophagus* from various parts of England, Scotland and Ireland.

R. melophagi closely resembles *R. quintana*, both in its morphology and method of multiplication. The organisms are especially abundant in the middle and hinder region of the stomach, where they often form a layer on the surface of the epithelium. It is difficult to be certain that they do not penetrate into the cells themselves, but the appearance of sections suggests that they only occur on the surface in the same way as *R. quintana*. The organisms pass to the outside together with the faeces and presumably the infection is thus transmitted from one *Melophagus* to another.

According to Sikora (1918) *Rickettsia melophagi* is transmitted hereditarily to the offspring of infected *Melophagus*, in which case it is evident that the organisms must have the power of passing through the tissues and invading the ovaries or testes. The examination of sections of the developing ova resulted in finding a few bodies that might have been *Rickettsia*, but when present in small numbers it is difficult to distinguish them with any certainty from artefacts and therefore the results were inconclusive. However, the presence of *Rickettsia* in the young offspring of infected *Melophagus*, renders it probable that this infection is hereditary.

The *Rickettsia* were frequently associated with *Crithidia melophagi* and occasionally a parasitic nematode was also found in the alimentary canal of the *Melophagus*.

Rickettsia trichodectae n. sp.

The examination of Mallophaga from the horse resulted in the discovery of *Rickettsia* in 7 to 8 per cent. of the lice examined. The species of Mallophaga was *Trichodectes pilosus* and the presence of a *Rickettsia* in these insects is especially interesting as they do not feed on blood, and therefore cannot acquire the infection by the ingestion of infected blood.

The parasites occur in the alimentary canal of the *Trichodectes* and closely resemble *Rickettsia melophagi* in their morphology. Their dimensions are about $0.3-0.5\mu$ in diameter by about 0.5 to 0.9μ in length and occasionally longer

forms may be observed. The organism multiplies in the alimentary canal and is passed out together with the faeces, thus furnishing a means for the spread of the infection from one insect to another. The name *Rickettsia trichodectae* is provisionally given to this parasite, for in spite of the absence of any distinguishing morphological characters, it can hardly be doubted that it represents a new species, in view of its occurrence in a distinct host.

Rickettsia linognathi n. sp.

The examination of smears from the alimentary canal of the goat louse, *Linognathus stenopsis*, resulted in finding *Rickettsia* in two examples out of 57 collected from goats at Cambridge. The parasite does not differ morphologically from the preceding species and as it was only found in smears of the contents of the alimentary canal it has not yet been possible to decide whether it occurs only in the lumen of the gut, or in addition in the epithelial cells. For convenience of reference the name *R. linognathi* is given to this organism, although as in the case of many other bacteria it is impossible to give satisfactorily distinguishing morphological characters.

EXAMINATION OF OTHER ARTHROPODA.

In the course of this investigation the gut contents of many species of blood-sucking insects, lice, Mallophaga and fleas have been examined and it is noteworthy how remarkably free from bacteria is the alimentary canal of most of these insects. Occasionally rod-shaped bacilli and sarcina like bodies were found, especially in the films made from lice, but as a rule they were absent.

In the case of *Haemotopinus suis* from the pig, most of the films were found to contain irregular-shaped bodies resembling the symbiotic fungi which occur in various species of insects, and especially in Hemiptera (Buchner, 1912).

DISCUSSION OF RESULTS.

Up to the present time nine species of *Rickettsia* have been recorded in addition to *Dermacentroxenus rickettsia* Wolbach (1918) which probably belongs to the same group of organisms. These different species and their habitats are summarised in the following table:

Rickettsia prowazeki da Rocha Lima 1916. Occurs in the lumen of the intestine and the epithelium of the mid-gut of *Pediculus humanus* and in the blood and organs of typhus patients.

R. quintana da Rocha Lima 1916. Occurs in the lumen of the alimentary canal of *Pediculus humanus* and in the blood and organs of trench fever patients.

R. pediculi da Rocha Lima 1917. Occurs in the lumen of the alimentary canal of *Pediculus humanus*.

R. melophagi Nöller 1917. Occurs in the lumen of the alimentary canal and the ovaries of *Melophagus ovinus*.

R. ctenocephali Sikora 1918. Occurs in the alimentary canal of *Ctenocephalus canis*.

R. trichodectae n. sp. Occurs in the alimentary canal of *Trichodectes pilosus*.

R. linognathi n. sp. Occurs in the alimentary canal of *Linognathus stenopsis*.

Rickettsia sp. Sikora 1918. Occurring in the alimentary canal of *Psokus* and *Ctenopsylla musculi*, respectively.

All the *Rickettsia* mentioned in the above list inhabit the alimentary canal of Arthropods, and in addition two of them also occur in the blood of man and are associated with human diseases.

The examination of their hosts shows that these organisms have been found not only in blood-sucking insects, but also in at least two species that do not feed on blood, viz. *Trichodectes* and *Psokus*.

In view of these facts, therefore, it seems probable that the *Rickettsia* represent a group of micro-organisms which primarily inhabit the alimentary canal of insects and other Arthropods. Some of them live saprophytically in the mid-gut, whilst others, such as *R. prowazeki*, have become true parasites and invade the tissues of their host.

As they multiply in the intestine, numbers of the *Rickettsia* would be continually passing to the exterior in the faeces and doubtless this is the way in which the infection is usually transmitted from one host to another in the absence of any intermediate vertebrate host. In the case of blood-sucking insects such as lice, any organisms that are voided are continually being introduced into the blood of the host either through the open wound caused by the bite of the insect itself, or through excoriations of the surface of the body from independent causes. Under these conditions therefore any such organisms would have the chance of becoming adapted to a parasitic mode of life in the vertebrate host.

With regard to the difficult question of the relation between *R. quintana* and *R. pediculi* it seems not unreasonable to assume that here we have an example of a *Rickettsia* normally inhabiting the intestine of the louse which is becoming adapted to a parasitic mode of existence in the blood of man.

The presence of *Rickettsia* in apparently normal lice has been recorded by many competent observers and their results are not satisfactorily explained by the assumption that in all these cases the lice have come either directly or indirectly from patients suffering from typhus or trench fever. It is more in unison with the results to accept da Rocha Lima and Töpfer's hypothesis that there is a species of *Rickettsia*, *R. pediculi*, which lives in the alimentary canal of the human louse and is non-pathogenic to man. On the other hand, the results of Arkwright, Bacot and Duncan (1918) as well as my own, have conclusively proved that some strains of lice are practically free from *Rickettsia*, but when fed on trench fever patients become infected with *R. quintana*, an organism that seems to be indistinguishable from *R. pediculi* except with regard to its physiological character of living in the blood of man.

To explain these results it is suggested that the human louse is liable to be infected with a non-pathogenic variety of *Rickettsia*, *R. pediculi*, which, under certain conditions, has acquired the capacity of living in the blood of man and

produced a pathogenic variety, for which it is convenient to retain the specific name *R. quintana*.

An interesting parallel may be observed in the case of parasitic flagellates. It is well known that these occur in the alimentary canal of a large variety of invertebrates and some species such as *Trypanosoma* and *Leishmania* have become adapted to living in the blood of vertebrates. Most of these flagellates inhabit the alimentary canal of blood-sucking insects, but, as in the case of *Rickettsia*, some are found in animals that never feed on blood and therefore cannot acquire the infection in this way. On the other hand the results of Laveran and Franchini (1914) and Fantham and Porter (1916) have shown that it is possible to infect vertebrates artificially by the inoculation of flagellates from various species of invertebrates, and there is no reason to suppose that the introduction of parasites into the body by natural methods would be any less successful.

In conclusion, I should like to express heartiest thanks to my friend Prof. G. H. F. Nuttall, F.R.S., for his continual assistance and helpful advice.

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THE ANATOMY AND BIOLOGY OF THE PARASITIC *APHELENCHI*.

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(With Figs. 1, 2 on Pl. VIII and Figs. 3-32 in Text.)

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I. THE PARASITIC SPECIES OF THE GENUS *APHELENCHUS* AND THEIR DISCOVERY.

A. fragariae Ritzema Bos, 1891.

A. ormerodis R.B., 1891. A doubtful species, possibly the young of *A. fragariae* (Marcinowski, 1908).

A. olesistus R.B., 1893.

A. phyllophagus n. sp.

DISCOVERY OF THE PARASITE.

Aphelenchus fragariae was first described by Ritzema Bos (1891) as causing a disease among strawberry plants in Kent; the plants had been sent to him by Miss Ormerod.

Nematoid worms were observed to cause disease in the leaves of *Begonia* by Worthington Smith (1890), in plants which came from Dunstable. In the following year Klebahn (1891) found *Aphelenchi* in blotched leaves of two species of fern (*Asplenium diversifolium* and *bulbiferum*) growing in a nursery in Bremen, and sent specimens to Ritzema Bos. The latter authority, having found the same species in these specimens and in *Begonia* leaves from England, described it under the name of *A. olesistus*.

II. DISTRIBUTION OF THE PARASITES, AND THE DISEASES CAUSED BY THEM.

A. fragariae. The disease caused by this species has been named strawberry bunch by Cobb (1891) (Blumenkohlkrankheit der Erdbeerpflanze, Ritzema Bos, 1891). It is characterised by stunting of growth in the length of the stem, and hypertrophy in breadth; the flowers abort, and fruit is not formed. Complete failure of the crop consequently results. It has been recorded from England, Scotland, Germany, and Norway (Marcinowski, 1908), and is doubtless much more widely spread.

A. olesistus since its discovery in *Asplenium* and *Begonia* has been found in many species of ferns and flowering plants. It attacks the leaves, causing large, sharply defined, brown blotches; the leaves finally wilt and fall off. Louis Mangin (1895) recorded the disease "de la Rouille" in Everlasting (*Helichrysum*) cultivated in the districts of Ollioules, Bandol, and St Nazaire, near Toulon. It causes great loss, as affected plants are unsaleable. A similar disease has been recorded from chrysanthemums by Atkinson (1891), from the United States by Sorauer (1901), Hofer (1901), Osterwalder (1904), and Molz (1909) from Switzerland and Germany. Ritzema Bos identified Sorauer and Hofer's nematode as *A. olesistus*. Sorauer (1902) described a serious epidemic in *Begonia* "Gloire de Lorraine," in which the plants were unsaleable. Osterwalder (1902) found the disease in flowering plants cultivated in the open air in Wadenswil and Zürich, in *Anemone japonica* and *Sylvestris*, *Ranunculus montana*, *Atragena alpina*, *Eryngium alpinum*, *Scabiosa silenifolia*, *Spiraea astilboides*, *Epipactis palustris* and others. It also occurs in *Coleus* and *Salvia* (Hofer, 1901) and in orchids (Marcinowski, 1908). Among ferns *Pteris ouvardi* and *cretica* have been found affected in addition to *Asplenium*.

Material used in the present investigation.

I received (1) specimens of strawberry plants containing *A. fragariae* from a correspondent in Ayrshire, (2) leaves of *Lygodium dichotomum* and *Lomaria ciliata* containing *A. olesistus* from the Royal Botanic Gardens, Kew,

through the kindness of Sir David Prain and Mr Arthur Hill, and (3) *Chrysanthemum* leaves containing the new species *A. phyllophagus*, from Messrs Tacon and Horwood, Cheshunt, Hertfordshire.

Regarding this disease in chrysanthemums, Messrs Tacon and Horwood write, that they have had this trouble for ten years at least; in one batch of pot chrysanthemums (Cheshunt White) one half were affected; they have been compelled to abandon certain varieties entirely, as they used to lose all their leaves.

Strawberry bunch and *Aphelenchus* leaf disease are therefore two widespread and destructive plant diseases, which cause considerable loss to the growers of fruit and flowers.

III. SPECIFIC DISTINCTNESS OF *APHELENCHUS FRAGARIAE* R.B., *A. OLESISTUS* R.B., AND *A. PHYLLOPHAGUS* N.SP.

A. fragariae and *olesistus*. Ritzema Bos distinguished his two species by their pathological effects on their hosts. Several points of anatomical distinction must have been observed under low powers of the microscope, but they are unsatisfactory. The tail of *A. fragariae* narrows suddenly behind the anus, that of *A. olesistus* does not.

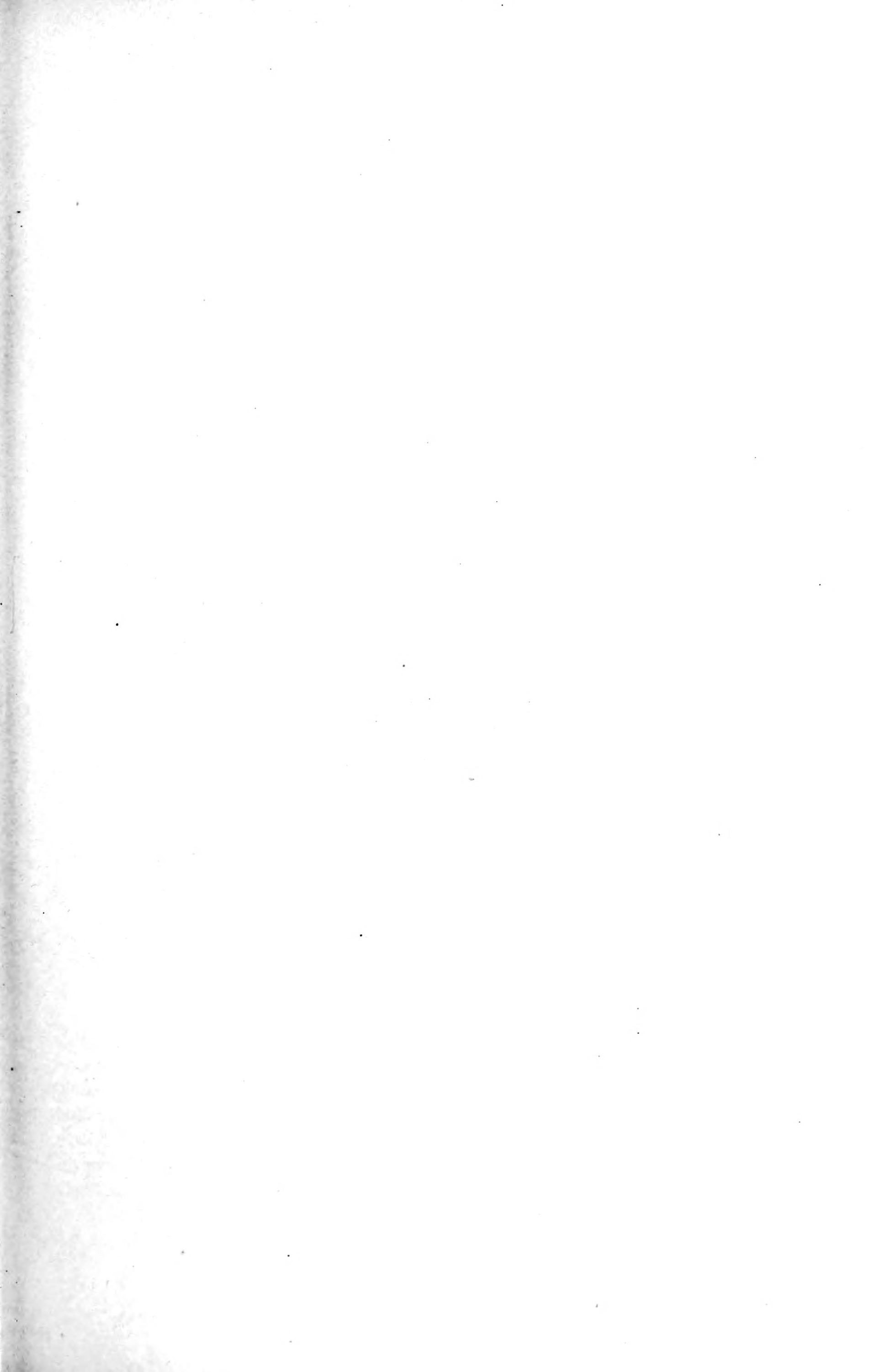
Marcinowski (1908) considers *A. fragariae* identical with *A. olesistus*, and possibly also with *A. helophitus* De Man. The differences in measurements she considers to be within the limits of individual variability. She performed experiments which she claims prove that the *Aphelenchi* of orchids and begonias can be transferred to strawberry plants.

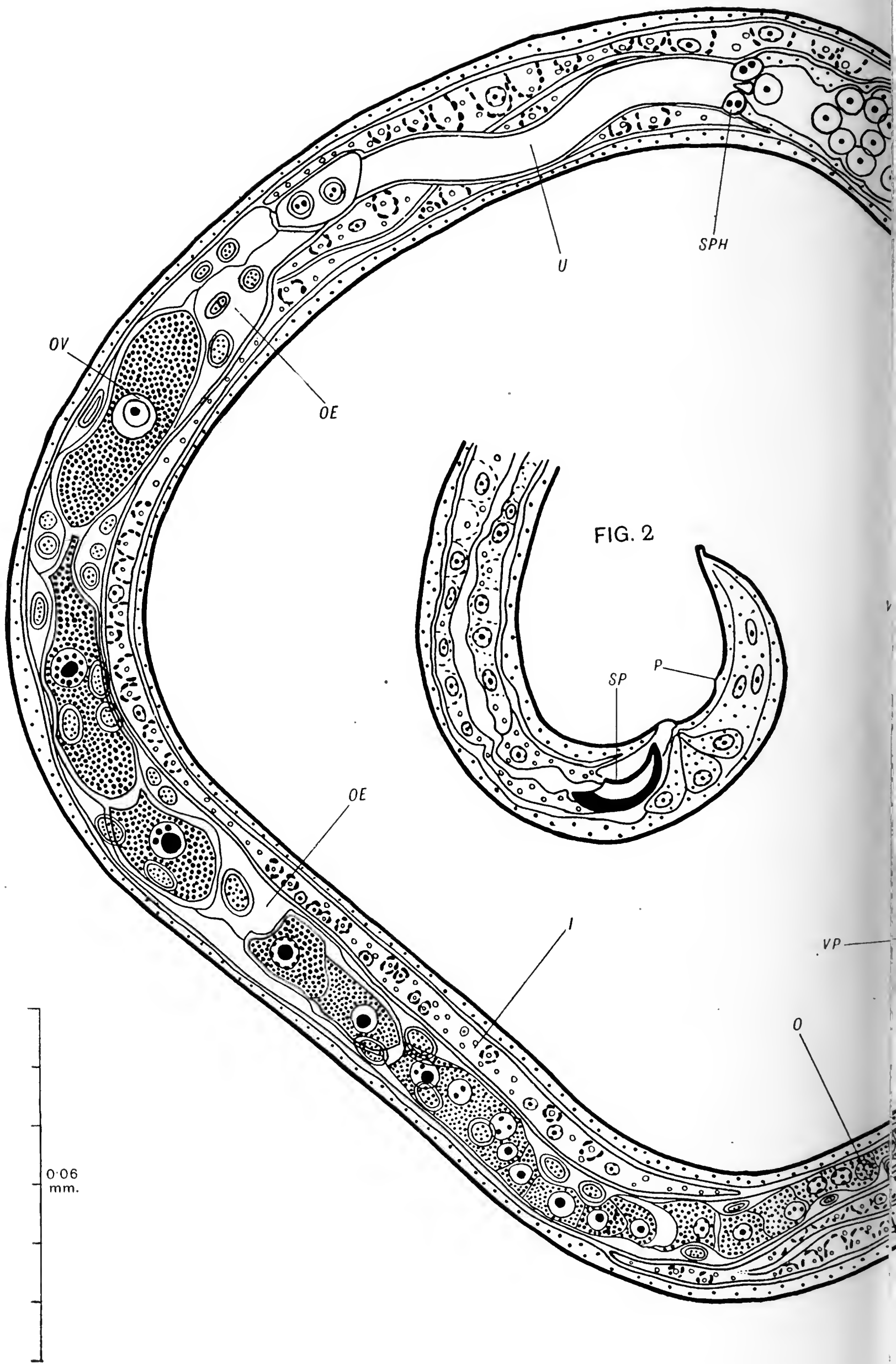
Infected orchid leaves were placed in contact with (1) begonias, (2) strawberry plants. In (1) immediate infection occurred with typical leaf disease; in (2) on the other hand no *Aphelenchi* were found on the strawberry plants during the first month, thereafter only a few in one plant; leaf disease and not characteristic strawberry bunch resulted.

This experiment therefore so far from proving the identity of *A. olesistus* and *fragariae* tends to prove their distinctness.

A. phyllophagus. When numerous fresh and well-mounted specimens are compared with *A. fragariae* and *olesistus* the following differences are obvious: it is a larger and more robust animal, and the excretory pore is situated some distance behind the nerve ring, while in *A. fragariae* and *olesistus* it is on a level with the nerve ring.

The difference in size between *A. phyllophagus* and *A. olesistus* is not a mere temporary difference between individuals of the same species due to the occupation of different host plants. I transferred both species to cabbage seedlings, but they retained their differences of size in the new host. It should be noted that the stomata (through which the parasites enter the plants, *vide* p. 176, E) are larger in the normal hosts of *A. phyllophagus* (chrysanthemums) than in the normal hosts of *A. olesistus* (begonias and ferns). The two species are therefore adapted to their normal hosts.





Figs. 1 and 2. *Aphaelion*
Fig. 1. Adult female from a chrysanthemum leaf; outlines drawn with a camera from a specimen stained with haemalum, and mounted

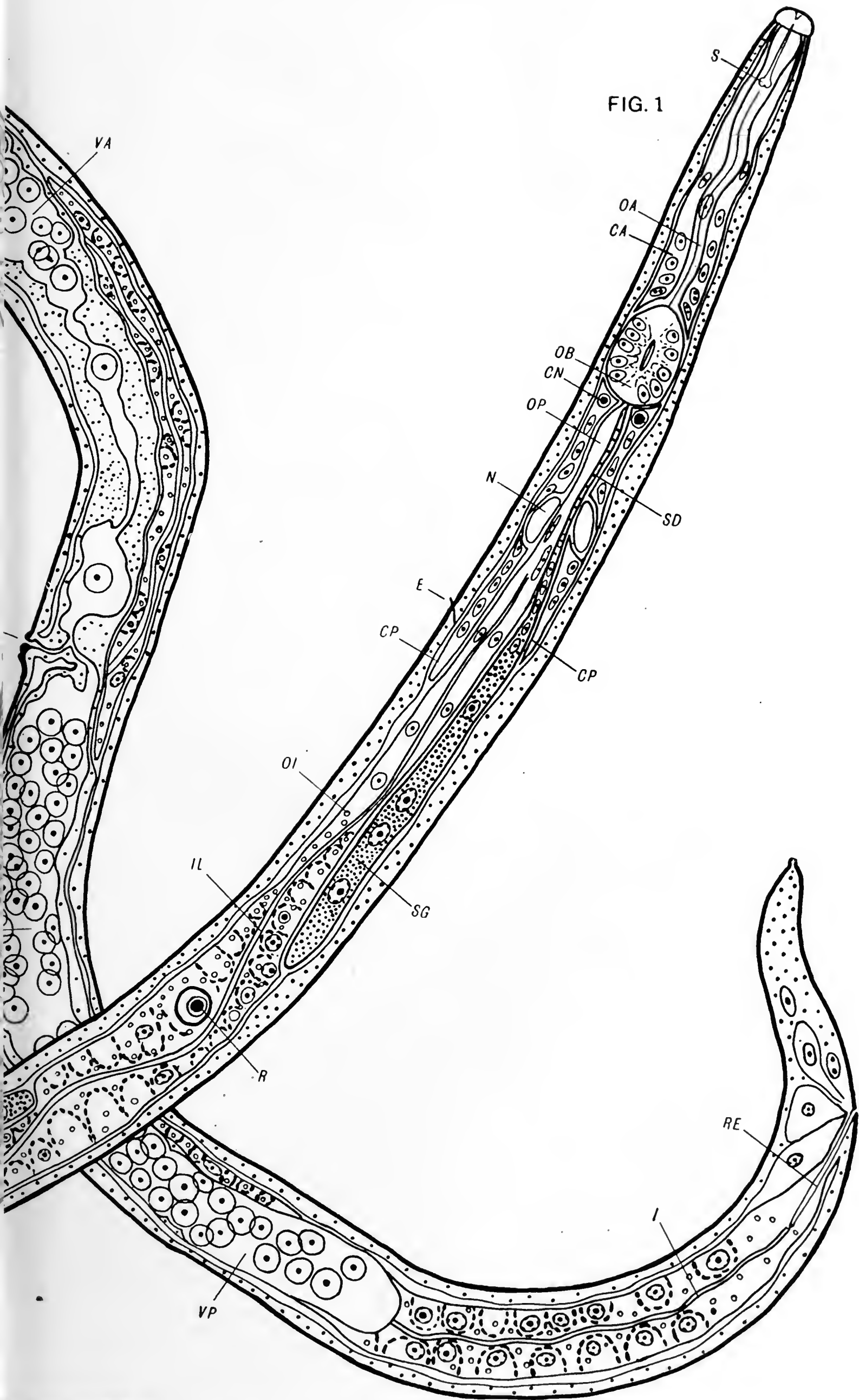


FIG. 1

hyllophagus n.sp.

with 70 per cent. alcohol, and mounted in glycerine jelly, detail completed from specimens

a. Fig. 2. Tail of male.

KEY TO THE GROUP OF THE SLENDER-BODIED *APHELENCHI*.

Aphelenchi of slender-body form. α^1 , 45 and over.

- (1) Excretory pore behind the nerve ring. Parasitic. L. 0.85–1 mm.
A. phyllophagus n.sp.
- (2) Excretory pore at the level of the nerve ring.
 - (a) Intra-uterine egg rounded oval. Free living. L. 1 mm. *A. helophilus* De Man, 1886.
 - (b) Intra-uterine egg elongated cylindrical. Parasitic.
 - (i) Anterior lip of anus prominent. Causes hypertrophy of tissues of host. L. 0.07–0.08 mm. *A. fragariae* R.B., 1891.
 - (ii) Anterior lip of anus not prominent. Does not cause hypertrophy. L. 0.05–0.06 mm. *A. olesistus* R.B., 1893.

IV. *A. PHYLLOPHAGUS* N.SP. ANATOMY OF THE ADULT.

Measurements. ♀, L. 0.845–0.92 mm.; α , 51–53 (β , 11.3–16.5)²; γ , 20.5–21. ♂, L. 0.88–0.96 mm.; α , 48–52 (β , 12.6–13); γ , 16–19.

Cobb's formula:	♀	1.3	(6.8)	9	?	70	95.5	0.923;
		1.2	(1.6)	1.8	?	2.1	1.3	
	♂	1.2	(7.4)	9.9	?	M	93	0.965.
		0.8	(1.4)	1.5	?	2	1.5	

Body-form (Figs. 1 and 2). The hemispherical *head* is marked off by a groove. *Lateral membranes* absent. *Transverse striae* of cuticle 0.0008 mm. in breadth. *Tail* ends in a mamilli-form appendage. *Lateral lines* (Figs. 4–14, *LL*) occupy $\frac{1}{6}$ th of the circumference. *Muscle fields* each contain five cells in cross-section.

Alimentary system. The *spear* (Fig. 1, *S*), length 0.013 mm., thickens slightly to the knobbed base. The *oesophagus* is divided into anterior oesophagus, bulb, and posterior oesophagus (Fig. 1, *OA*, *OB*, *OP*); the bulb is conoid and muscular; the intrinsic nuclei form a layer in its surface; the centre is occupied by the usual oval chitinous structure; the posterior oesophagus³ is swollen slightly behind the bulb, narrows as it traverses the nerve ring, then increases gradually in width to its junction with the intestine, which is marked only by the commencement of the intestinal droplets and granules (Fig. 2, *OI*); the wall of the oesophagus is eosinophil in staining reaction, its lumen circular in the posterior section. The *salivary glands*³ (Figs. 1, 11 and 12, *S.G.*) lie dorsal to, and slightly to the right of the posterior oesophagus and the commencement of the intestine; they consist of four cells arranged in linear series: the duct (Figs. 1 and 6–10, *S.D.*), containing several nuclei, passes forward through the nerve ring, and enters the oesophageal bulb at its posterior end (Fig. 5, *S.O.*); both gland and duct are basophil.

¹ De Man (1886) employs the following useful contractions:

$\frac{\text{Length}}{\text{Breadth}}$	α .	$\frac{\text{Length}}{\text{L. of oesophagus}}$	β .	$\frac{\text{Length}}{\text{L. of tail}}$	γ .
	Length, L.		Maximum breadth, B.		

² The length of the oesophagus is measured throughout to the posterior end of the bulb, owing to the indefiniteness of the function of the oesophagus and intestine. This measurement is given as the second figure in Cobb's formula—in brackets. The formula therefore runs:

Posterior end of spear, (Posterior end of bulb), Nerve ring, ?, Vulva, Anus.

³ Cf. p. 169 (1).

The *intestine* (Figs. 1, 13 and 14, *I*) is thick and patent; walls without cell limits but containing numerous nuclei, droplets and granules; staining reaction basophil; the lumen is sinuous, flattened and slit-like, contrasting with the round lumen of the posterior oesophagus. The *rectum* (Fig. 1, *RE*) is one half to two-thirds as long as the tail.

Nerve ring and oesophageal collar. The *nerve ring* (Figs. 1, 9 and 10, *N*) is situated behind the oesophageal bulb at a distance rather greater than the length of the bulb. The *oesophageal cellular collar* (Figs. 1 and 6–12, *CA*, *C.P.*) is an ingrowth from the four longitudinal lines (Fig. 8, *C.P.*) and clothes the oesophagus throughout its whole length; it contains many nuclei, of which two or four situated immediately behind the bulb (Figs. 1 and 6, *CN*) are particularly large and prominent; the function of the collar is probably nervous, it, together with the nerve ring, representing the central nervous system of the animal.

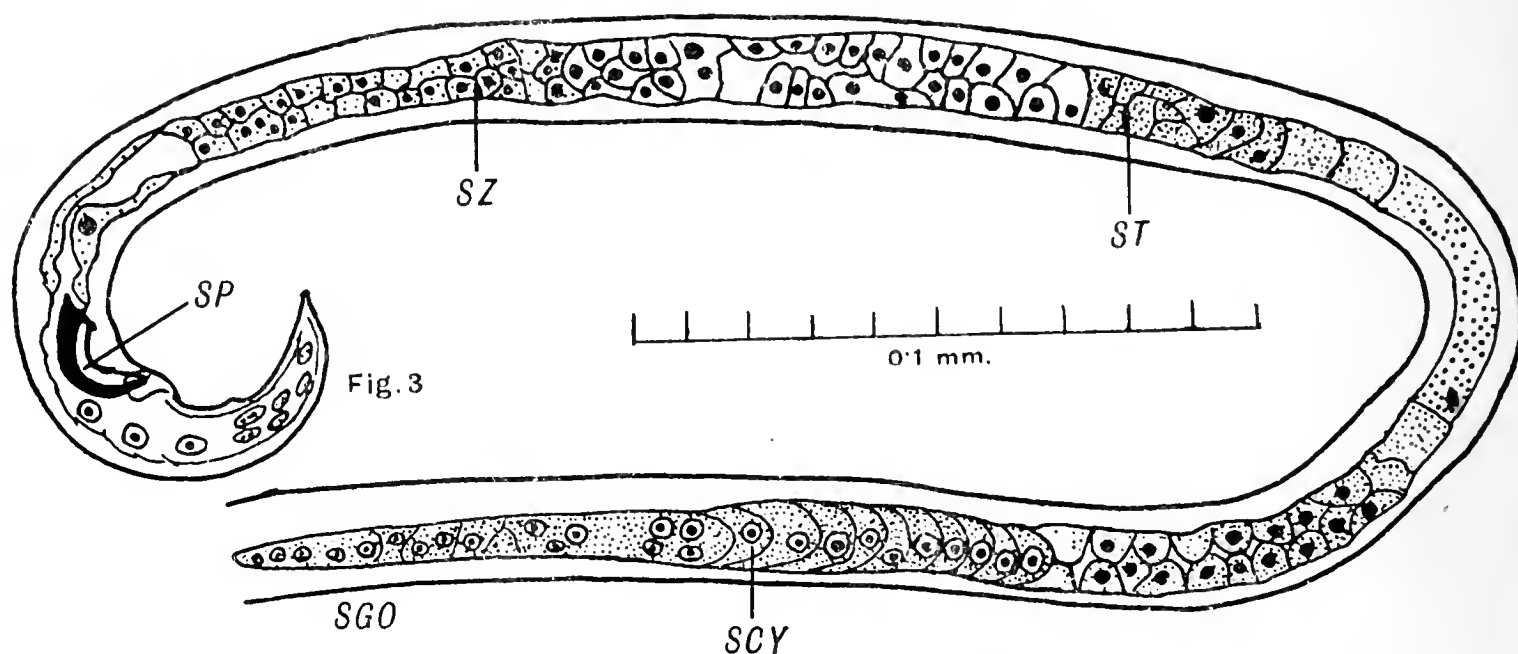
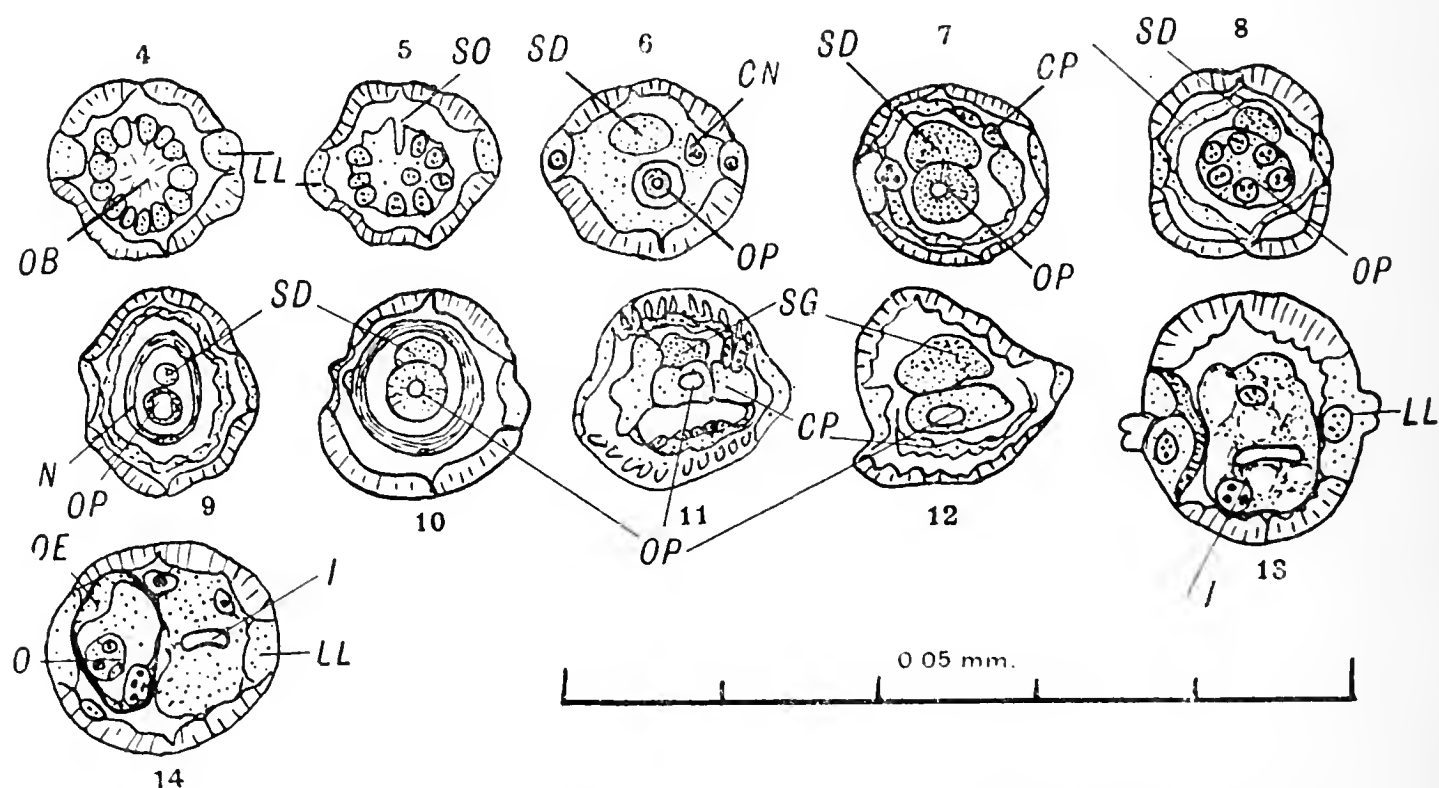


Fig. 3. *Aphelenchus phyllophagus* n. sp. Posterior two-thirds of male, showing gonads.



Figs. 4–14. *Aphelenchus phyllophagus* n. sp. Transverse sections in series.

Figs. 4, 5. Through the oesophageal bulb.

Figs. 6–8. Between the bulb and the nerve ring.

Figs. 9, 10. Through the nerve ring.

Figs. 11, 12. Between the nerve ring and the end of the oesophagus.

Fig. 13. Through the intestine in front of the gonad.

Fig. 14. At the commencement of the ovary.

Excretory system. The single *renette*¹ cell (Fig. 1, *R*) lies between the left lateral line and the intestine, at a level midway between the end of the salivary gland and the commencement of the gonad; it is somewhat difficult to recognise; in unstained preparations it has the appearance of a spherical space containing a small highly refringent sphere, which in stained specimens proves to be a chromatin mass. I have not succeeded in tracing the duct between the renette cell and the excretory pore; this aperture is situated behind the posterior margin of the nerve ring at a distance varying slightly in different specimens, but averaging 0.02 mm., equal to rather more than the length of the oesophageal bulb; this position markedly behind the nerve ring is of systematic importance (*vide supra*); the excretory duct contains a substance which stains with haematoxylin, giving a curious appearance, as if a spine were projecting through the cuticle.

There are no *caudal glands*.

The reproductive system in the female consists of two tubular sacs (Figs. 1 and 14, *OE*, *VA*, *VP*) extending forward and backward from the vulva; the anterior end of the anterior sac contains the ovary (Figs. 1 and 14, *O*); the ova (Fig. 1, *OV*), as they pass backward increase in size and become elongated oval in shape; the wall of this part of the sac consists of a fairly robust endothelium, which, in those places where it contains nuclei, bulges into the lumen and compresses or separates the ova (Fig. 1, *OE*); the nuclei have a characteristic circumvallate appearance. The structure of the *uterus* (Fig. 1, *U*) is difficult to analyse; it is closed at its posterior end where it joins the anterior vagina by a sphincter (Fig. 1, *SPH*). It is probable that fertilisation and formation of the shell occur in the *anterior vagina*. Marcinowski (1910) describes a shell gland in *Cephalobus*, but it should be remembered that Schneider (1866, p. 285) proved that the shell of nematode eggs is secreted by the egg itself, and not by the wall of the oviduct. The anterior vagina extends from the sphincter to the vulva; its walls are of irregular thickness, and do not contain the typical nuclei of the ovarian sac; the lumen is occupied by a mass of spherical spermatozoa. The retrovulvar portion of the reproductive tube consists of the *posterior vagina* alone, which resembles the anterior vagina in length and other respects, but is a caecum; the vaginae function as receptacula seminis. (For development in the larva, and early fertilisation, see Sect. V below: for comparative anatomy, p. 172 (3).) The *vulva* is a transverse slit situated $\frac{7}{10}$ ths of the body-length from the head.

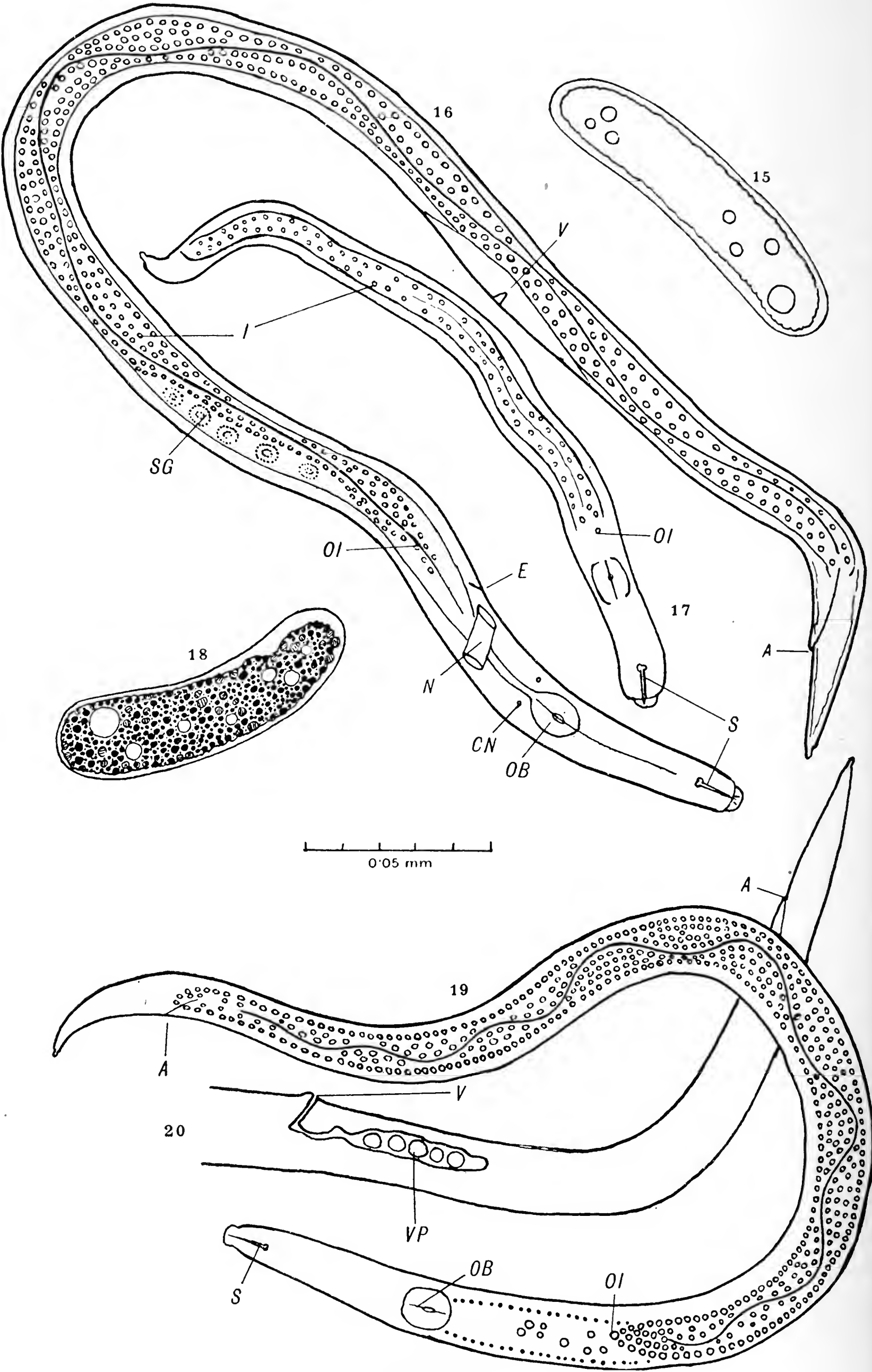
Male reproductive organs (Figs. 2 and 3). The single testicular tube has an endothelial wall, like the ovarian; posteriorly it joins the intestine to form the cloaca, and is here closed by a valve-like projection of the ventral wall. The *spicules* (Figs. 2 and 3, *SP*) are very broad; curved and hollow, the posterior margin is much thickened, the anterior margin is the structure described and figured by Ritzema Bos (1893) as the accessory piece; the two spicules are very closely apposed; there is no accessory piece. The *tail of the male* (Fig. 2) is sharply curved, the ventral surface flat, with one poorly-defined papilla, post-anal, median ventral (Fig. 2, *P*); in mounted specimens there is sometimes an appearance of a bursal membrane, which is probably artificial, but the flattening of the ventral surface must produce a ridge along each lateral line which forms the rudiment of a bursal membrane.

V. A. PHYLLOPHAGUS. THE EGG, AND LARVAL DEVELOPMENT.

The *egg* (Figs. 15 and 18) is sausage-shaped, 0.085×0.023 to 0.095×0.022 mm. The shell is thin and unsculptured, the ovum when first laid unsegmented. I have not observed segmentation or embryonic development.

Larvae. Molz (1909) gives the length of the newly-hatched larva as 0.16–0.18 mm. and its breadth 0.01 mm. (α 16–18). The youngest larva which I have seen (Fig. 17) measured 0.22×0.015 mm. (α 14.7), the spear 0.012 mm.; it was found in the leaf axil of a groundsel on the 28th day after infection, together with adults and eggs, and had recently hatched

¹ Cf. p. 172 (2).



Figs. 15-20.

in that situation. An older larva (Fig. 19) measured 0.465×0.02 mm. (a 23), spear again 0.012 mm.; it was found in the tissue of a leaf of a groundsel on the 5th day after infection, together with larvae measuring 0.25 , 0.3 , and 0.32 mm. Being thus associated with recently hatched larvae it was probably derived from an egg laid within the previous five days in a leaf axil of the plant. In both the 0.22 mm. and the 0.465 mm. larva the oesophageal bulb was distinct; the intestine (Figs. 17 and 19, *OS*) commenced behind the bulb at a distance equalling head to bulb; the alimentary canal was patent.

A still older larva (Fig. 16) measured 0.65×0.018 mm. (a 36); spear still 0.012 mm.; the *salivary gland* (*SG*) was apparent, with five nuclei of which the anterior probably forms the duct; the gland was situated further back than in the adult, the whole of it lying behind *OI*; the nerve ring (*N*), and the two large nuclei of the posterior collar (*CN*), were also apparent, the latter as two black dots which I at first took for eye spots; the excretory pore was in the same situation as in the adult. The rudimentary vulva was situated three-quarters of the body-length from the head; it lay in a clear area in the midventral line, which represented the rudiment of the vaginae. This specimen was found in a leaf of a groundsel, ten days after infection, together with adults and other larvae. A young female (Fig. 20), from the same leaf, was probably also hatched in the same situation, not more than ten days previously; it measured 0.8×0.022 mm. (a 36).

The outstanding fact in this specimen was the condition of the developing genital ducts, the vulva and the posterior vagina only being present, the latter already containing spermatozoa! The rudiment of the ovary was not observed, but there was no trace of germinal cells at the fundus of the vagina; a condition of protandrous hermaphroditism was therefore not present, such as occurs in *Rhabditis* (*Leptodera*) (Schneider, 1866, p. 316).

Length of time required for embryonic and larval development, and by one generation from egg to egg.

We have seen above that recently hatched larvae, measuring 0.25 – 0.46 mm., have been found in the leaf of a plant exposed to infection for only five days, older larvae, 0.65 , and young adults after only ten days. We may therefore conclude that embryonic development does not occupy more than five days, complete embryonic and larval development not more than ten, and that a generation from egg to egg could be completed in fourteen days, the conditions of temperature being those of a European spring, summer or autumn, with the day temperature not falling below 15° C. (60° F.). This agrees with the observations of Ritzema Bos (1892) on *Tylenchus dipsaci* (*devastatrix*) Kühn. As in this species, and in contrast to *T. tritici*, many generations can thus succeed each other in the course of one year, enormous multiplication may occur in a short period.

Figs. 15, 16. **Aphelenchus phyllophagus** n. sp. Specimens from *Senecio vulgaris*.

Fig. 15. Egg from leaf axil, 28th day. Outline.

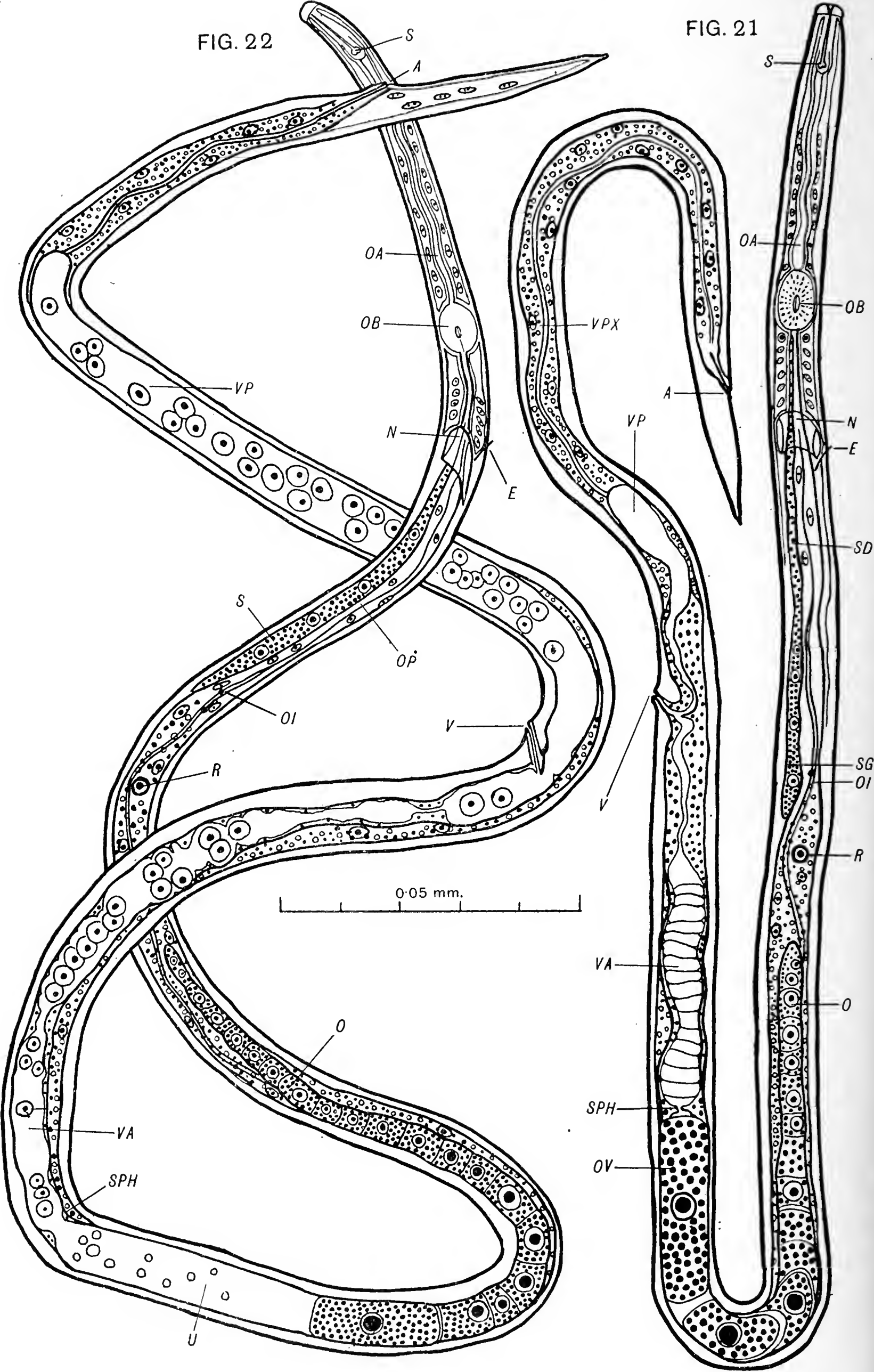
Fig. 16. Larva, 0.65 mm., from mesophyll, 10th day.

Fig. 17. Larva, 0.22 mm., from leaf axil, 28th day.

Fig. 18. Egg from leaf axil, 28th day.

Fig. 19. Larva, 0.465 mm., from mesophyll, 5th day.

Fig. 20. Young female, 0.8 mm., posterior third of body, from mesophyll, 13th day.



VI. *A. OLESISTUS* R.B. 1893. ANATOMY. (Figs. 21, 23, 24.)

Measurements. ♀ L. 0.529 mm., B. 0.011 mm., a 48, (β 8), γ 20. ♀ L. 0.574 mm., a 44, (β 11), γ 19. ♂ L. 0.5 mm., a 50, (β 8.87), γ 15.

$$\text{Cobb's formula:} \quad \begin{array}{cccccc} \text{♀} & 1.8 & (9) & 11.8 & ? & 70 & 95 \\ & 1 & (1.8) & 2 & ? & 2.3 & 1.2 \end{array} \quad 0.574.$$

In general anatomy, this species, as well as *A. fragariae*, so strongly resembles *A. phyllophagus*, that it is necessary to refer to a few points only. *Cuticular striae* are not visible. *Spear*, 0.01 mm. *Excretory system*, the renette cell (*R*) lies in the same position on the left side; the excretory pore (*E*) is however at the level of the nerve ring. *Reproductive system: female*, the structure is naturally more difficult to distinguish than in the larger species; in the specimen figured the uterus is occupied by a large cylindrical egg without a shell; the sphincter at the junction of uterus and anterior vagina is well developed; the spermatozoa occupying the anterior vagina are arranged in a rouleau. (The posterior vagina extends further back than is shown in Fig. 21, to the point marked *VPX*.)

Male spicules (Fig. 24). Length, 0.013 mm.; no accessory piece, no caudal papilla.

VII. *A. FRAGARIAE* R.B. 1891. ANATOMY. (Figs. 22 and 25–29.)

Measurements. ♀ L. 0.723 mm., B. 0.012 mm., head to posterior margin of bulb, 0.065 mm.; tail, 0.038 mm., a 60, (β 11), γ 19.

$$\text{Cobb's formula:} \quad \begin{array}{cccccc} \text{♀} & 1.6 & (9) & 11 & ? & 69 & 93 \\ & 0.8 & (1) & 1.1 & ? & 1.6 & 1 \end{array} \quad 0.723.$$

This is the most slim of the three species, especially as regards the oesophageal region of the body. Compare breadth at the nerve ring, 1.1 per cent. with 2 per cent. in *A. olesistus*.

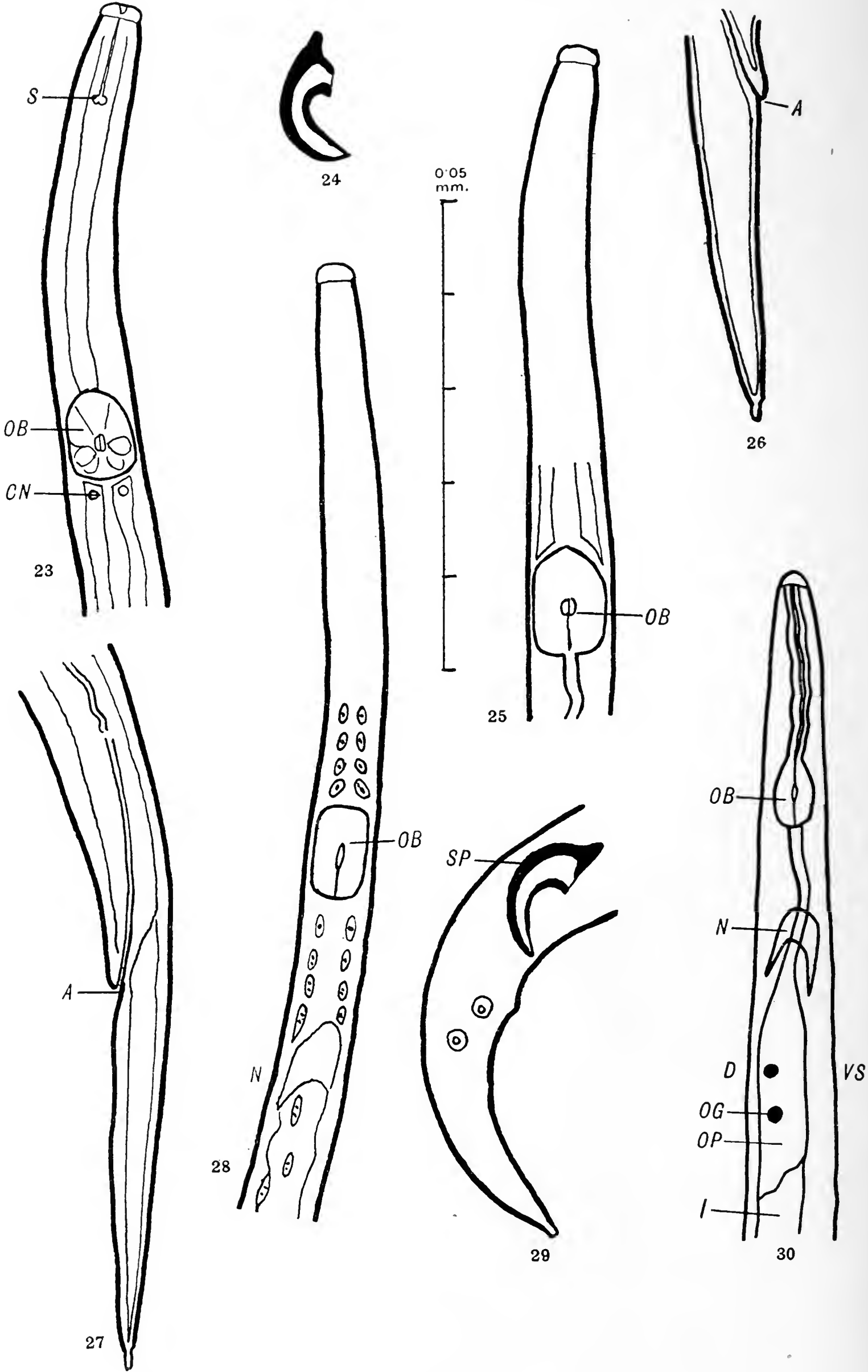
Spear, 0.01 mm. *Oesophageal bulb* conoid to cylindrical (Figs. 25, 26). *Rectum* (Fig. 27), long, nearly equal to the length of the tail. The anterior lip of the *anus* is prominent (Figs. 27, 28). *Excretory pore* at the level of the anterior margin of the nerve ring. *Female reproductive system*: the uterus is well-defined (Fig. 22, *U*), and is marked off from the anterior vagina by the sphincter (*SPH*); the anterior and posterior vaginae are of equal length. *Spicules* of the male (Fig. 29), 0.014 mm.; no accessory piece, the accessory of Ritzema Bos being again the anterior margin of the spicule.

VIII. COMPARATIVE ANATOMY OF (1) THE OESOPHAGUS AND SALIVARY GLANDS, (2) THE RENETTE, (3) THE VAGINAE, IN THE GENUS *APHELENCHUS*.

1. THE OESOPHAGUS AND SALIVARY GLANDS. In this genus the *oesophagus* is usually described as terminating at the bulb, and as joining the intestine at that point. The following facts, however, prove that the section of the alimentary canal between the bulb and the point of commencement of the intestinal droplets and granules (Figs. 1, 16–19, 21 and 22, *OI*) is morphologically the posterior oesophagus: (1) it is embraced by the nerve ring; (2) it is

Fig. 21. *A. olesistus* R.B., adult female from cabbage seedling infected 14 days previously from *Lomaria ciliata*. Fixed in Bouin's sol., mounted in glycerine jelly. (Note: The posterior vagina extends to the point *VPX*.)

Fig. 22. *A. fragariae* R.B., adult female from bud of rawberry plant; fixed in Bouin's sol., stained with haemalum, and mounted in balsam.



Figs. 23-30.

clothed by the oesophageal cellular collar; (3) its walls are eosinophil in contrast to the basophil intestine; (4) its lumen is cylindrical, that of the intestine is flattened. The lack of a definite line of demarcation from the intestine is due to the separation of the oesophageal glands from the body of this portion of the oesophagus, which will be considered in the next paragraph.

The *salivary glands* have been described in *Tylenchus similis* Cobb, by that writer (1915). They resemble those of *Aphelenchus phyllophagus*, *olesistus*, and *fragariae*. (Cobb has, however, traced their duct through the substance of the bulb and anterior oesophagus to an opening at the base of the spear.) The posterior oesophagus in *Tylenchus similis* also resembles that of the *Aphelenchi*, and differs from the corresponding organ of other *Tylenchi*, e.g. *T. dipsaci* Kühn, in which it is thick, club-shaped, and glandular, and is sharply marked off from the intestine (Fig. 30). In *T. dipsaci* this posterior bulb contains several large nuclei in its dorsal wall, while no salivary glands are present. It is therefore a reasonable supposition that the posterior oesophageal bulb of the *Tylenchi* (less *T. similis*) represents, morphologically and physiologically, the combined posterior oesophagus and salivary glands of *Aphelenchus* and *T. similis*; in other words, the oesophageal glands of *Tylenchus*, which are situated in the dorsal sector of the posterior bulb, have, in *Aphelenchus* and *T. similis*, separated themselves from the oesophagus to form the salivary glands.

Considering further the anatomy of the oesophagus and its glands, in other nematode genera, we find three glands, one in each longitudinal sector of the organ, which open by three ducts into the alimentary canal (*Thoracostoma* and *Cylicolaimus* Jägerskiöld, 1901; *Oncholaimus* Stewart, 1906; *Ascaris*, adult, Jägerskiöld; *Agchylostoma* Looss, 1911). The dorsal gland is, however, always more important than the two subventrals, is longer, and stains more deeply (Jägerskiöld, 1901, p. 14). Finally on examining the larva of *Ascaris lumbricoides* (Stewart, 1921), we find that these three glands originate from a single giant nucleus in the hind end of the dorsal sector, which, as growth proceeds, expands downward into the subventral sectors. We therefore have in series (1) *Aphelenchus* and *T. similis* with the glands separate from the oesophagus, on its dorsal surface, as the "salivary glands"; (2) *Tylenchus* (less *T. similis*) and the larvae of *Ascaris* to the seventeenth day, with

Figs. 23, 24. **A. olesistus** R.B.

Fig. 23. Male, outline of oesophageal region.

Fig. 24. Male, spicule.

Figs. 25-30. **A. fragariae** R.B.

Fig. 25. Male, outline of oesophageal region.

Fig. 26. Female, outline of tail.

Fig. 27. Female, outline of tail.

Fig. 28. Female, outline of oesophageal region.

Fig. 29. Male, outline of tail.

Fig. 30. **Tylenchus dipsaci** (**devastatrix**) Kühn, from clover, fixed in 70 per cent. alcohol, mounted in glycerine jelly.

the glands in the dorsal sector; (3) *Ascaris* larva of the nineteenth day with glands growing from the dorsal sector into the two subventrals; (4) the great majority of adult nematodes with the typical three oesophageal glands.

2. THE RENETTE lies between the left lateral line and the intestine in *Tylenchus similis* Cobb (1915), and the three species under consideration. In the development of *Ascaris lumbricoides*, it originates in the same position, and thence gives rise to the apparently bilateral organ of the adult Ascarids. This origin of the excretory cell from the left lateral line is striking, and may prove to be of very general occurrence. Without laying too much stress on the homology, it may be recalled that the great, unicellular, skin glands of both lateral lines are the excretory organs of *Cylicolaimus magnus* Villot, *Thoracostoma acuticaudatum* Jägerskiöld (Jägerskiöld, 1901), and of the adult female of *Oncholaimus vulgaris* Bast. (Stewart, 1906).

3. THE VAGINAE are derived, as we have seen (p. 167) from a superficial cell group, which gives rise to them only, and not to the remainder of the reproductive tube—the gonad proper. They are probably ectodermal, their walls are not endothelial, and their junction with the uterus is marked by a sphincter. The corresponding sphincter is situated at the junction of the vagina and uterus in *Cylicolaimus*, *Thoracostoma* (Jägerskiöld, 1901), and *Oncholaimus* (Stewart, 1906), in which genera the vaginae are much shorter than in *Aphelenchus*. In *Cylicolaimus* and *Thoracostoma* sperm is permitted to pass the sphincter, and the uteri function as receptacula; in *Aphelenchus* the vaginae are large enough to accommodate the whole sperm mass; in *Oncholaimus*, where sperm is not allowed to pass into the uterus, although the vagina is too small to contain it, the surplus is drained off into the intestine by the gonenteric canals (Stewart, 1906).

The posterior vagina is the only portion of the posterior reproductive tube (of e.g. *Tylenchus*) which persists in *Aphelenchus*.

IX. BIOLOGY OF THE PARASITIC APHELENCHI.

The following experiments and observations were made principally on the species *A. phyllophagus* and *olesistus*. It may, however, be assumed that the three species are indetical in their general mode of life.

A. DEFINITIVE HABITAT OF THE PARASITE.

Aphelenchus fragariae. The strawberry plants received by Ritzema Bos (1891) were not fresh, and he was therefore not able to make satisfactory observations on the situation of the parasite. Marcinowski (1908) found them to be mainly ectoparasitic, in the leaf axils, and in the flowers among the stamens, during the month of May; only a few were endoparasitic, in brown patches of leaf sheaths. In June, however, they were chiefly endoparasitic, in leaves and stem. She notes that apparently healthy runners contained *Aphelenchi* in the bud.

In specimens sent to me in January I found adult and larval *A. fragariae*, in large numbers as ectoparasites under the scales of the bud. In serial sections (Fig. 32), they are seen lying among the hairs on the surface of the growing point.

The definitive habitat of *A. olesistus* and *phyllophagus*, that in which feeding and reproduction proceed most actively, and in which their pathological effects are manifest, is in the mesophyll spaces of leaves; they are never

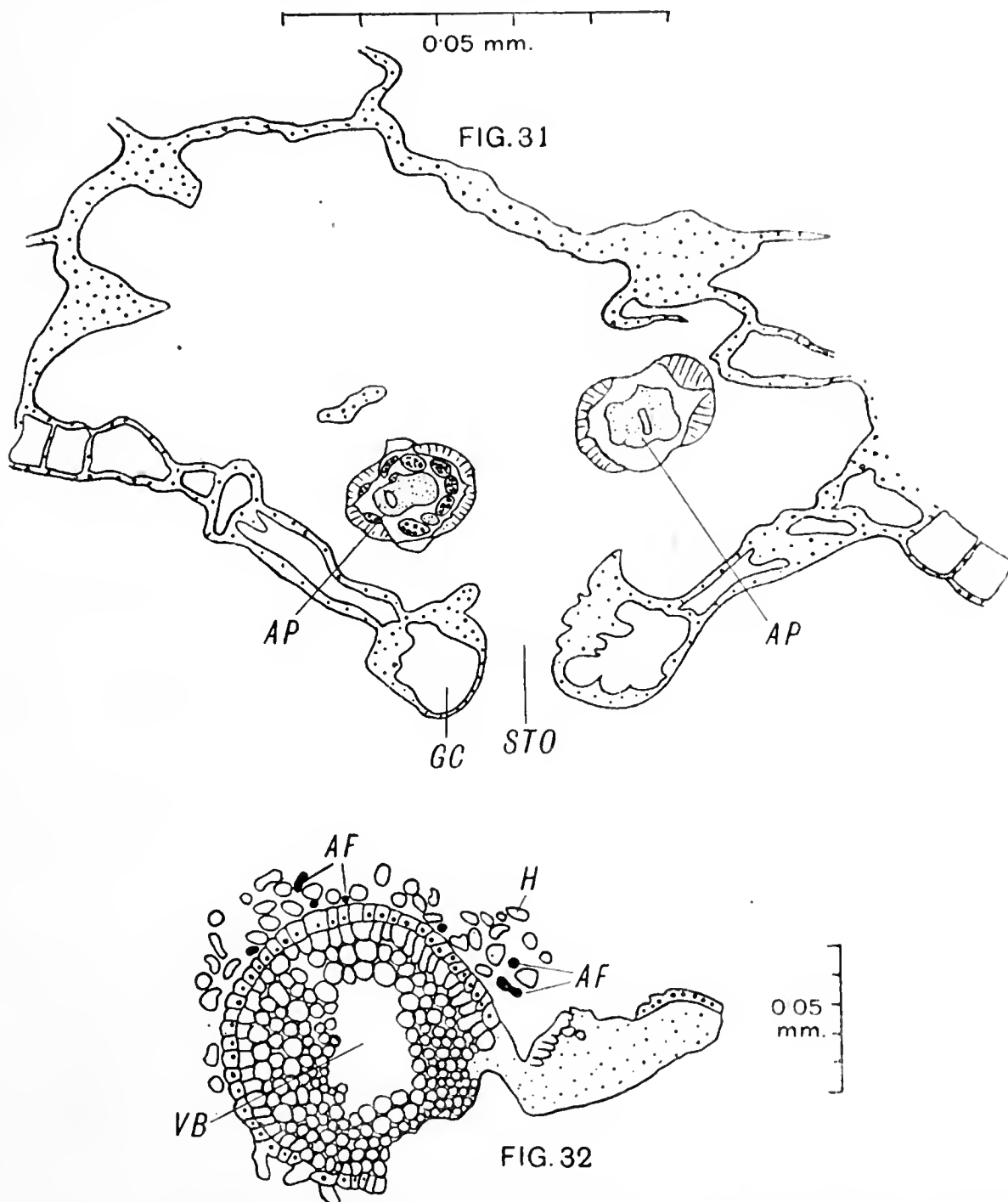


Fig. 31. Chrysanthemum leaf, transverse section through a stoma, showing *A. phyllophagus* in a mesophyll space.

Fig. 32. Strawberry plant, transverse section through the bud, showing *A. fragariae* on the surface.

intracellular, and avoid tissues without spaces (Ritzema Bos, 1893; Molz, 1909); they occur frequently close to the stomata.

MIGRATION IN THE TISSUES OF THE LEAF. Ritzema Bos (1893) believes that *A. olesistus* travels in or along the outside of the nerves. Molz (1909) denies this, and asserts that migration only occurs through the spaces of spongy tissue, and on the surface; he finds the vessels of chrysanthemum leaves to be too narrow to admit the parasite; the animal wanders out of the

diseased patches in search of fresh nourishment for itself and its progeny; eggs are found laid only in healthy tissue, consequently the gravid female has the greatest impulse to travel.

Adults and larvae *reach the soil* by the fall of the diseased leaves, bringing the life cycle of the race¹ to the resting stage in the soil.

B. THE RESTING STAGE IN THE SOIL.

Marcinowski (1910) asserts that the parasitic *Aphelenchi* can live only for a very short period in the soil, in fact that they only enter the soil in order to pass directly from a fallen leaf to a new host plant.

She placed portions of infested leaves on the earth of a pot, which was kept damp, and examined the earth from the second week onward—result negative. On the other hand, earth in which an infested *Begonia* had grown, proved infectious when placed on leaves of begonias.

Ritzema Bos (1892) showed that *Tylenchus dipsaci* can persist in the soil only on the surface, where it can undergo partial drying, and so enter a dormant condition. I found that adult *A. phyllophagus* can live actively in water for at least six days. Early death in damp soil may be due to mycosis.

Molz (1909) apparently believes that *A. phyllophagus* resides in the soil.

I have made the following observations to test the power of survival in earth:

Portions of infected leaves of *Chrysanthemum* and *Lomaria ciliata* were placed on earth in pots, which were watered daily, the surface of which, however, remained dry except for a period not exceeding two minutes each day. On the 7th and 9th days many adults, male and female, were found on the surface of the leaf fragments, a few actually in their tissues.

On the 16th, 17th, 20th and 46th days the surface layer of earth was examined. It was quite dry at the time, and had been at a temperature below the freezing point at night. Adult *Aphelenchi* were found in large numbers in a dormant condition, but they resumed activity after immersion in water. Larvae and eggs were looked for on the 46th day, but were not found. In earth taken from 10–15 mm. below the surface no *Aphelenchi* were found.

We can therefore conclude that *A. phyllophagus* and *olesistus* reaching the soil in fallen leaves, can live there for at least 46 days. Like *T. dipsaci*, they collect on the surface of the earth, where they suffer partial desiccation, and pass into a condition of suspended vitality. In the case of *T. dipsaci*, it is the larvae only which survive in this manner, while in *Aphelenchus* on the contrary the adults survive, and it is doubtful whether the larvae do so (they probably grow to adults before abandoning the fallen leaves).

¹ It should be clearly realised that the life cycle in *Aphelenchus* and *T. dipsaci* is that of the race, while in *T. tritici* it is that of the individual; in other words in the cycle from (a) the definitive habitat, through (b) the resting stage in the earth, and (c) the stage of immigration, many generations are completed in the former, only one generation in the latter; also many cycles can be completed in one year in the former, only one in the latter. Hence the enormously greater power of multiplication in the former than in the latter.

We now proceed to the consideration of:

C. THE ROUTE TAKEN BY THE PARASITE ON THE HOST DURING
IMMIGRATION FROM THE SOIL.

There are two views on this subject: (1) that the parasite, having entered the host plant below the level of the ground, ascends through the tissues of the stem to the leaves (Ritzema Bos, 1893, and Marcinowski, 1908-1910); (2) that it enters through the surface of the leaf, having reached this situation by travelling over the surface of the plant.

(1) Ritzema Bos (1893) found *Aphelenchi* in apparently healthy leaf-stalks of *Begonia* and *Asplenium*. In an experiment with an infected *Pteris*, he cut off all the parts of the plant above ground; the young fronds which grew up contained *Aphelenchi*, and he concludes that they came from the interior of the rhizome; it is, however, equally probable that they came from the surface of the soil. Marcinowski (1908) found a few *Aphelenchi* in the stem of a plant. The two observations of *Aphelenchi* in the stem appear to have been made by dissection or teasing; in such a method it must be borne in mind, that worms lurking on the surface may appear in the medium, and it may be thought that they have issued from the tissues. I have found a few *Aphelenchi* on teasing up portions of flower stalk, but on cutting serial sections have never found them actually in the stalk or stem; in one case I divided a groundsel stem longitudinally in halves, one half was teased out in water, and numerous adults and larvae found, the other half was cut into serial sections, but no *Aphelenchi* were found in the tissues, although several appeared on the cut surface.

The following observations also are offered to this view: the parts of an infected plant below the leaves are healthy (Klebahn, 1891); plants placed among infected leaves, if protected by a ring of vaseline around the stem, remain free of the parasite (Marcinowski, 1908); if all diseased leaves are removed from a plant, the buds growing out of the axils remain free (Molz, 1909).

We can therefore conclude that *Aphelenchi*, in their invasion of the host plant, do not traverse the tissues of the stem.

APHELENCHI in the soil, revived by moisture, and attracted by a suitable plant, wander on to it, and may live for some time as ectoparasites in the leaf axils before reaching their definitive habitat in the mesophyll.

In order to trace the course of immigration, I placed a number of young plants of groundsel (*Senecio vulgaris*) in pots of earth, on the surface of which infected leaves of *Chrysanthemum* and *Lomaria* had been strewn. The pots were kept in a room at a temperature of 10-15° C. (50-60° F.), were freely exposed to the sun, and were watered once daily. Entire plants or leaves were examined at intervals with the following results:

2nd day. Four entire plants negative.

3rd day Three plants examined. Plant (a): on syringing out the leaf axils with water three adult males and four females were found; in a leaf touching the ground—one adult female. The earth in this case had been infected 44 days previously, and had been bare of vegetation for some hours before the planting of plant (a). Plant (b): in a leaf axil one adult, on a leaf one larva, 0.305 mm. long. The earth had been infected 43 days previously, a chrysanthemum which bore ectoparasitic *Aphelenchi* was growing in the same pot. Plant (c): negative.

5th day. Lower leaves from four plants. (a), (b) and (c) were negative. (d): in the tissues of one lower leaf four larvae found. 0.25, 0.3, 0.32, 0.465 mm. long; soil infected 45 days previously; the leaf axils were unfortunately not examined, but it is probable that they contained adults which were the source of these larvae, a chrysanthemum was however also growing in the pot. 10th day. In a low growing leaf many adults and larvae found; the pot had been infected 41 days previously, and had been bare of vegetation for three days before the planting of this specimen, the adults must therefore have lived at least three days, and almost certainly 31 days in the soil before attacking this plant. 8th, 13th and 26th days. Adult *Aphelenchi* in the leaf axils or lower leaves. 28th day. Adults, larvae and eggs found in the leaf axils of the lower 20 mm. of the stem, adults and larvae also in the tissues of the lower leaves.

Marcinowski (1910) with begonias planted in infected soil, found that leaves in contact with the earth were invaded.

We can therefore conclude that adults invading a plant from the soil may take up ectoparasitic life in the leaf axils up to a height of at least 2 cm. above ground, and may here deposit eggs; the larvae hatched from them then proceed to the more extended invasion, and to their definitive habitat in the mesophyll. On the other hand, leaves close to the ground level may be invaded direct from the soil, the worms becoming entoparasites forthwith.

This brings us to the passage from ecto- to entoparasitic life, and the mode of entry into the spaces of the mesophyll:

D. *APHELENCHI* HAVING REACHED THE LEAF SURFACE ENTER THE MESOPHYLL SPACES THROUGH THE STOMATA.

This statement rests on the following observations: (1) *Aphelenchi* from orchid leaves, dropped in water on the ventral surface of leaves of an inverted *Begonia*, entered the leaves; application of infected to healthy leaves gave the same result (Marcinowski, 1908). (2) *Aphelenchi* have been observed traversing the stomata (Osterwalder, 1902 [quoted by Marcinowski], Marcinowski, 1908).

Molz (1909) held that the worms did not enter through the stomata, but through wounds of the plant surface, on the ground that (1) the stomata were too small to admit the parasites, and (2) leaf to leaf infection succeeded only with infected leaves. On the other hand, (1) my measurements of *Chrysanthemum* stomata give larger apertures (viz. 0.02×0.01 mm., and the shorter diameter can be increased by the forcible abduction of the guard cells), and the size of the parasite varies with the size of the stomata (*vide* bottom of p. 162); and (2) the negative result of Molz's leaf to leaf infection is explained by conditions of atmospheric humidity (Marcinowski, 1910, *vide infra* (E)).

E. INFLUENCE OF ATMOSPHERIC HUMIDITY, AND OF PREVIOUS DISEASE OR INJURY OF THE HOST PLANT, IN ASSISTING MIGRATION OF THE PARASITE. IMMUNITY.

Marcinowski (1908, 1910) records three important experiments on the influence of damp on the migration of *Aphelenchi*. (1) A portion of infected chrysanthemum leaf was mounted, ventral surface uppermost, in water on a slide; many worms were observed to creep out through the stomata. (2) If infected plants are placed in a humid atmosphere the parasites wander out, and adopt ectoparasitic life in the leaf axils. (3) Leaf to leaf infection experiments in begonias succeed in a humid atmosphere under a bell jar, but fail in the open air.

When chrysanthemum leaves are moistened by heavy dew, adult *Aphelenchi* can be observed (under a binocular microscope) wandering freely over their hairy surface (Molz, 1909).

High humidity assists migration by approximating the conditions of life on the plant surface to those prevailing in the mesophyll spaces. It is not, however, essential to migration (*vide* p. 175, and since *Aphelenchus* disease prevails in the open air).

Sorauer (1902) maintains that *Aphelenchi* cannot invade healthy plants, that a disease of the vascular bundles is produced in pot plants grown at a temperature above the optimum, and that *Aphelenchi* enter such diseased plants only. Marcinowski (1908), on the other hand, infected healthy begonias in a natural manner.

Aphelenchi are attracted by cell sap. I found that they collect in considerable numbers in plant wounds. Molz's theory of entry through wounds is doubtless true in a limited sense; the injuries to which he refers are caused by insect parasites, and by the eruption of axillary buds.

Certain races of many species of flowering plants are immune to the parasite (see *litt. passim* and Molz, 1909).

X. METHODS OF COMBATING THE DISEASE.

Chemical treatment is of use only in such plants as Everlasting (Mangin, 1895), if the blotching appears after the harvest, when the flowers are stored in warehouses. Exposure to a dry atmosphere saturated with carbon bisulphide for 24–48 hours, kills the parasites without affecting the appearance of the plants. In no other plants has chemical treatment of any kind been found of avail, since chemicals of sufficient strength damage the appearance of the plant.

Treatment by heat. Marcinowski (1910)—immersion of the plant in water at 50–52° C. for five minutes, kills the parasite but not the plant.

Prophylaxis based on the habitat of the parasite in the surface of the soil. Earth for filling pots or boxes should be dug from a pit, surface earth being rejected. For open air culture deep ploughing of infected fields may prove useful. All infected leaves and plants should be burned. If it is necessary to use infected stock plants, they should be treated by the hot-water method, or by burying to a depth of at least three inches—after daily watering for at least a week, the surface earth should be removed, after which it is unlikely that any *Aphelenchi* will remain on the plant.

XI. TECHNIQUE.

Fixatives. Carnoy's chloroform-acetic-alcohol mixture, Schaudinn's or Bouin's fluid, and boiling 70 per cent. alcohol are satisfactory; the first-named gives the most clear definition.

Stains for mounting *in toto* haemalum differentiated with acid 70 per cent. alcohol, following Carnoy or Schaudinn is best; after Bouin, picrocarmine gives the best stain.

Mounting. Looss' alcohol-glycerine evaporation method, followed by glycerine jelly for unstained specimens. For stained specimens transfer to xylol, and add canada balsam very gradually on several successive days.

Embedding in paraffin. The tissue should be transferred to chloroform, paraffin (60° C. M.P. for vegetable tissues) added to saturation, the chloroform slowly evaporated in an incubator for 24 hours. Transfer to two baths of melted paraffin for 5 and 10 minutes respectively.

Staining sections. Thionin and eosin give the best results.

SUMMARY.

There are three species of true parasites in the genus *Aphelenchus*, viz.: *A. fragariae* R.B., 1891, *A. olesistus* R.B., 1893, and *A. phyllophagus* n.sp. *A. ormerodis* R.B., 1891, is a doubtful species, possibly a young form of *A. fragariae* (Marcinowski, 1908).

A. fragariae causes the disease strawberry bunch; *A. olesistus* and *phyllophagus* cause leaf disease in flowering plants and ferns. The diseases are widely distributed and of considerable economic importance.

From the study of the anatomy of the three species certain points of general importance emerge: (1) The salivary glands of *Aphelenchus* represent the oesophageal glands of other nematode genera, which have separated from the body of the oesophagus. This separation causes the reduction in size of the posterior portion of the oesophagus, and its lack of clear demarcation from the intestine. (2) The excretory organ of *Aphelenchus*, and of some other nematode genera, originates from the left lateral line. (3) The vaginae are ectodermal organs distinct in origin from the gonads proper.

The life cycle of the parasitic *Aphelenchi* is divided into three definite stages: (A) That of residence in the definitive habitat, which for *A. fragariae* is in the stem and leaves of the strawberry plant, for *A. olesistus* and *phyllophagus* in the mesophyll spaces of the leaves of many plants. Nutrition and reproduction are most actively carried on in this situation, several or many generations succeeding each other, and here the pathological effects are manifest. (B) The resting stage in the soil. The worms reach the soil in fallen leaves, and the adults survive in a partially dried, dormant condition on the surface of the soil for prolonged periods. (C) The stage of immigration into the host plant. When revived by moisture the *Aphelenchi* may be attracted by a suitable plant, and wanders on to it; they may live as ectoparasites in the leaf axils, breeding in this situation, the larvae migrating to the definitive habitat; or they may enter the definitive habitat direct. They do not traverse the tissues of the stem during immigration. *A. olesistus* and *phyllophagus* enter the mesophyll spaces through the stomata.

It should be noted that this life cycle is that of the race, not of the individual. It comprises many generations, and in this respect resembles that of *Tylenchus dipsaci* and differs from that of *T. tritici*, which includes one generation only.

In the life of the individual *Aphelenchus*, embryonic development occupies not more than five days, embryonic and larval development not more than ten, and a complete generation not more than fourteen days.

High atmospheric humidity assists the migration of the worms, previous disease or injury of the host plant may do so. Some races of various species of plants are immune.

Treatment of the affected plants by chemical methods is of limited applicability. Treatment by immersion in water at 50–52° C. for five minutes is recommended by Marcinowski (1908). Prophylaxis should be based on burning of infected plants and leaves, and on the avoidance of infected surface soil in the filling of pots and boxes.

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A, anus; AF, *Aphelenchus fragariae*; AP, *A. phyllophagus*; CA, anterior section of oesophageal collar; CN, collar nucleus; CP, posterior limit of collar; D, dorsal surface; E, excretory pore; GC, guard cell of stoma; H, hair; I, intestine; IL, intestinal lumen; LL, lateral line; N, nerve ring; O, ovary; OA, anterior oesophagus; OB, oesophageal bulb; OE, endothelium of gonad tube; OG, oesophageal gland; OI, junction of oesophagus and intestine; OP, posterior oesophagus; OV, ovum; P, papilla; R, renette cell; RE, rectum; S, spear; SCY, spermatocyte; SD, salivary duct; SG, salivary gland; SGO, spermatogonium; SO, opening of salivary duct into bulb; SP, spicule; SPH, sphincter; ST, spermatid; STO, aperture of stoma; SZ, spermatozoon; U, uterus; V, vulva; VB, vascular bundle; VA, anterior vagina; VP, posterior vagina; VS, ventral surface.

SUPPLEMENTARY ACCOUNT OF THE DIPTEROUS LARVAE FEEDING UPON MOLLUSCS.

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IN my previous paper (*Parasitology*, XI. 1919) on the life history of a Calliphorine fly, *Melinda cognata* Meig., the larvae of which live as parasites in a snail (*Helicella virgata*), I gave a complete account of all the Dipterous larvae feeding upon molluscs.

Since the publication of my paper several new records dealing with this subject have been published or communicated to me by letters. As some of this information is yet unpublished while the rest is scattered in different journals it may be useful to bring together these records and thus to complete the list of observations dealing with the Dipterous fauna of molluscs.

1. *Melinda cognata* Meigen.

Soon after publication of my paper on the life history of this fly, Sir Arthur Shipley wrote a popular account of it, which appeared in *Country Life*, January 3rd, 1920, pp. 14-15. In reply to this article he received from E. Adrian Woodruffe-Peacock a very interesting letter which he forwarded to me. This correspondent writes that he has often observed *Melinda cognata* depositing its eggs on *Helicella virgata*. He has often met with the larvae of this fly and he believes that the huge mortality of *Helicella* observed by him in 1917 was undoubtedly due to them.

2. *Sarcophaga filia* Pandellé.

An interesting account of the life history of this fly has been recently given by J. Rostand (1920). This author found in July of 1916 and 1917 at Camb (Basse-Pyrénées, France) a large number of a small snail (*Helix*) harbouring Dipterous larvae. The adult flies bred from these larvae were identified by Dr Villeneuve as *Sarcophaga filia* (Rond.) Pandellé. According to Rostand the larvae are real parasites since he found them in their young stages attacking almost healthy snails. He compares this case of parasitic life to that of *Melinda cognata*. The larva of *S. filia* completely devours its host and is transformed into the pupa inside the emptied shell of the snail. 15 days later the adult fly emerges. He thinks that there is only one generation *per annum*.

The larvae of *S. filia* are often parasitised by Hymenoptera belonging to the families of Braconidae and Cynipidae.

3. *Sarcophaga melanura* Meigen.

M. E. Séguy informs me that he has observed the females of this fly depositing their larvae upon the slug *Arion fuscus*. In spite of the violent contractions of the slug and the abundant production of mucus the larvae make their way into the body of the host which is soon killed and liquefied.

4. *Engyzops pecchiolii* Rond.

A recent paper of W. R. Thompson (1921) contains much interesting information concerning this fly. He dissected a female of *E. pecchiolii* captured in the south of France (Gers) and found its internal genital organs well developed and the uterus filled with completely formed larvae ready to hatch from their eggs. The study of these first stage larvae revealed their close resemblance to the first stage larva of *Melinda gentilis*, which, as we have previously shown (1919, p. 442), lives as a parasite in *Helicella virgata*. The genus *Engyzops* was placed by previous authors among the sarcophagid flies, but according to Thompson the structure of the internal genital organs and the first stage larva show that this insect has more affinity with the Calliphorine genus *Melinda* than with the Sarcophagids.

The life history of *E. pecchiolii* is not yet known, but the resemblance of its first stage larva to that of *Melinda* suggests, according to Thompson, the possibility that in its early stages it also lives on snails.

5. *Lucilia dux* and *Pycnosoma*.

Under the title "Mortality among snails and appearance of Blue-bottle Flies," we find in *Nature* (1919, civ. pp. 412-413) a very interesting letter by N. Annandale of the Indian Museum, Calcutta. The following are a few extracts from this letter:

"The residential parts of Calcutta are remarkably free, as a rule, from both house-flies (*Musca* spp.) and blue-bottles. This is doubtless due to the excellence of the municipal sanitary arrangements, for at Sibpur, a few miles away, blue-bottles (*Pycnosoma* or *Lucilia dux*) are not only extremely troublesome in the houses, but are also probably connected with frequent epidemics of enteric, unknown in the better parts of Calcutta. For some years past I have been able to trace the flies to their breeding-ground. This has always been the dead bodies of the snail *Achatina fulica*, the largest land mollusc in Bengal...." "Fortunately it is largely a feeder on decaying vegetable and animal matter, and therefore does little harm to crops or gardens, and has even its value as a scavenger. Since, however, I found the maggots in the dead snails, I have noticed that the appearance of blue-bottles in this part of Calcutta invariably coincides with a heavy mortality in the mollusc, which appears to be subject occasionally to some kind of fatal epidemic and also

perishes in large numbers after egg-laying at the beginning of the rainy season and during dry spells in and at the end of that season....”

6. *Sciomyza dubia* Flin.

In his report on land and fresh water Mollusca observed in Hertfordshire, C. Oldham writes (1912, p. 288): “Among some Mollusca collected in February on a hedge bank at Tring, I found dead shells of *Vitrea rogersi*, *V. nitidula*, *V. cellaria* and *Pyramidula rotundata*, each of which had in its last whorl a small reddish pupa. From these several specimens a dipteran, *Sciomyza dubia*, hatched out in April. Almost nothing is known of the life-history of this fly, and I cannot say whether the larva is parasitic on the living Mollusc, kills and subsequently devours it, eats the body of the snail which has died from some independent cause, or merely chooses the empty shell as a convenient place in which to pupate.”

In a letter to me, dated 26. xii. 1919, C. Oldham mentions that in the autumn he often finds on the chalk downs near Berkhamstead, snails (*Helicella* and others) which harbour fly larvae of an undetermined species; these larvae are sometimes seen free in the collecting tubes containing the snails.

7. Phoridae.

Several species of Phorids which usually inhabit the dead snails have been recently carefully studied by Lundbeck (xii. 1919) and as a result of his study the following changes have to be introduced in my previous list of these inhabitants of snails: the *Phora* sp. No. 2, the pupae of which I have found in *Helix nemoralis* collected in the Bernese Oberland, Switzerland (1911, p. 60, and 1919, p. 450), have been identified by Lundbeck as *Phora* or *Paraspini-phora notata* Zett.

Phora bergenstammi Mik. The Phorid, the complete life history of which I described under this name (1911, pp. 31–56, and 1919, p. 449), was proved by Lundbeck to be *Phora domestica* Wood (1906).

Phora domestica Wood was considered by Malloch (1910) to be synonymous with *Ph. bergenstammi* Mik., and this was accepted in 1912 by Wood himself. Lundbeck has proved, however, that these two species, although closely allied, differ in several respects.

Phora bergenstammi Mik. has “4 dorsocentral bristles in both sexes, and the male palpi almost nude”; “the costa (in both sexes) reaches beyond the middle of the wing, the first costal division is at most double as long as the second, and the fourth vein is rather strongly curved at the base.”

Phora domestica Wood has “4 dorsocentral bristles in the female and 2 in the male and the male palpi with normal armature...”; “the costa in both sexes does not reach quite to the middle of the wing, the first costal division is more than double as long as the second and the fourth vein is less curved at the base; further, *domestica* seems always to have pale hind margins to the abdominal segments not present in *bergenstammi*.”

As to the puparia of these two species they also differ: "By examination of the puparia of *domestica* sent me from Dr Schmitz it was proved, that these puparia are identical with the puparium described by Keilin (*Bull. Sci. France et Belgique* (7), 45, 1911, 47) as *bergenstammi*, so that the species treated by Keilin under that name is in reality *domestica*. An easily observable difference between the two puparia is, that while that of *domestica* has at the lateral margin of each segment two papillae, a small and a larger, with bristles on the apex, there are in *bergenstammi* on each segment three such papillae, a small, a medium sized and a large (Fig. 2)."

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NOTE: Professor A. E. Boycott, F.R.S., has kindly called my attention to the following few misprints which he found in my previous paper (1919):

- pp. 433 and 435, instead of *Hygromyia* read *Hygromia*.
- p. 434, para. 2, and in References, instead of *Steep* read *Step*.
- p. 435, para. 1, instead of *rufiscence* read *rufescens*.
- p. 442, para. 5, and p. 448, para. 2, instead of *cantioniformis* read *cantianiformis*.

He informs me also that "*H. virgata* is hardly 'very common' in England and Wales. In many parts of the west and north it is quite a rare species, especially inland."

ON THE NEPHROCYTES OF *PEDICULUS HUMANUS*.

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(With 5 Text-figures.)

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INTRODUCTION.

DISSECTIONS of the thorax of *Pediculus humanus* ♂, ♀, or larva, demonstrate the existence of two groups of special cells lying suspended on either side of the oesophagus between the reniform salivary glands and the forwardly protruding intestinal caeca. These cells have already been observed by entomologists who have studied the anatomy of the louse; Patton and Cragg (1913, p. 559, Pl. LXX, figs. 1 and 2) were the first to see and figure them. These authors refer to them as follows:

“Embedded among the fat body, immediately anterior to the kidney-shaped glands, there is on each side a small collection of round cells, readily distinguished from the fat body by their more glistening appearance. These are constant in position, and are always found, in sections and dissections, surrounding the oesophagus, though not attached to it in any way. They have no capsule, but appear to be connected with one-another by short and pointed processes. The protoplasm of these cells is vacuolated, and contains many fine granules. One, or sometimes two, nuclei are present, and appear as clear vesicles containing a darkly staining mass of chromatin. No duct has been distinguished with certainty, though in some dissections a fine

filament which may perhaps be a duct has been noted passing upward with the salivary duct."

These cells were also observed by Müller (1915, p. 15), who described them in a few lines in his chapter on the fat body. The only interesting statement he makes is that these cells owing to their size, shape and occurrence in groups, recall the pericardial cells of other insects but differ therefrom, however, in respect to their position.

Sikora (1916, pp. 57-58) subsequently gave a good description, calling them "large celled glands" ("Grosszellige Drüsen"). She studied them in sections and found that there were 46-56 cells ("Drüsen") on each side of the oesophagus, although she stated that the number may be much smaller because it was possible that she counted some of the cells twice over. She found that the cells are binucleate and frequently show cleft-like spaces ("spaltförmige Safräume (?)") between their nuclei. The cells are oval and often prolonged into a filament; other very fine filaments connect them to each other and serve to attach them to the surrounding organs. Sikora found no efferent canal connected with the cells and supposed that their function consists in taking up certain substances from the perivisceral fluid, either storing, transforming, or eliminating them again into the perivisceral fluid. She compares these cells to the binucleate cells found by her in the vicinity of the heart in transverse sections of *Haematopinus eurysternus*.

The foregoing paragraphs afford a summary of all that has been published hitherto regarding the structure of these cells.

In the course of our studies on *Pediculus*, we have been able to confirm the statements of the above-cited authors regarding the structure of the cells in question. We have, however, found cells of a similar structure in other parts of the body of the louse and have succeeded in determining the hitherto unknown function of all these elements.

For reasons to be given presently, we shall henceforth refer to these binucleate (at times trinucleate) elements as *Nephrocytes*. The elements referred to by the above-cited authors will be termed by us *peri-oesophageal nephrocytes* (ventral, in clusters), the others will be termed *disseminated nephrocytes* (dorsal).

Peri-oesophageal Nephrocytes in Pediculus.

The number of nephrocytes belonging to this category is certainly inferior to what Sikora supposed, for we have been unable to count more than 16 to 20 on each side of the oesophagus. In sections the nephrocytes appear as large binucleate or at times trinucleate cells possessing very granular protoplasm (Fig. 1). The cleft-like spaces of Sikora appear to be artefacts due to fixation, since they are not visible in the living tissue. The nuclei are vesicular, and, like the nuclei in the other tissues of the louse, they are very poor in chromatin. The chromatin is reduced to a few granules, the rest of the nucleus being clear.

The nephrocytes, when examined alive (Fig. 2), are seen to contain numerous droplets of variable size, which are of a greenish colour. These droplets are larger and more numerous in fasting lice. The size of the droplets at times exceeds that of the nucleus, which may consequently be obscured. The droplets are not dissolved and not decolourized by 80 per cent. alcohol or by weak acetic acid.

The nephrocytes are linked to each other by very fine connective filaments and *they have no duct or the like which communicates outwardly or with the alimentary canal.*

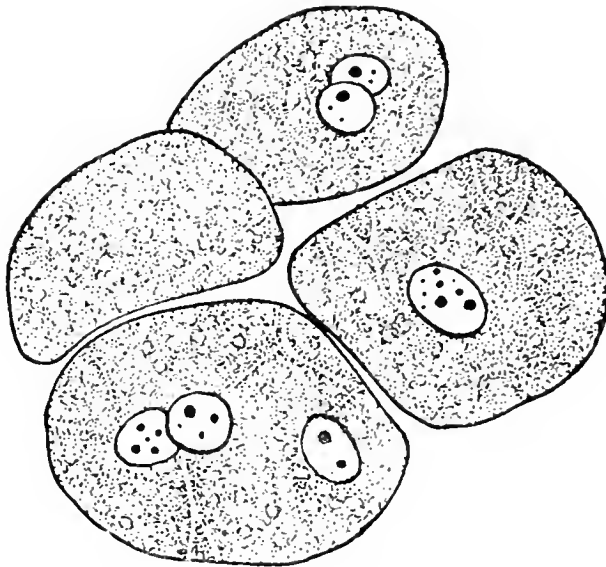


Fig. 1. Peri-oesophageal nephrocytes of *P. humanus* in section (fixed in Carnoy, stained by iron-haematoxylin).

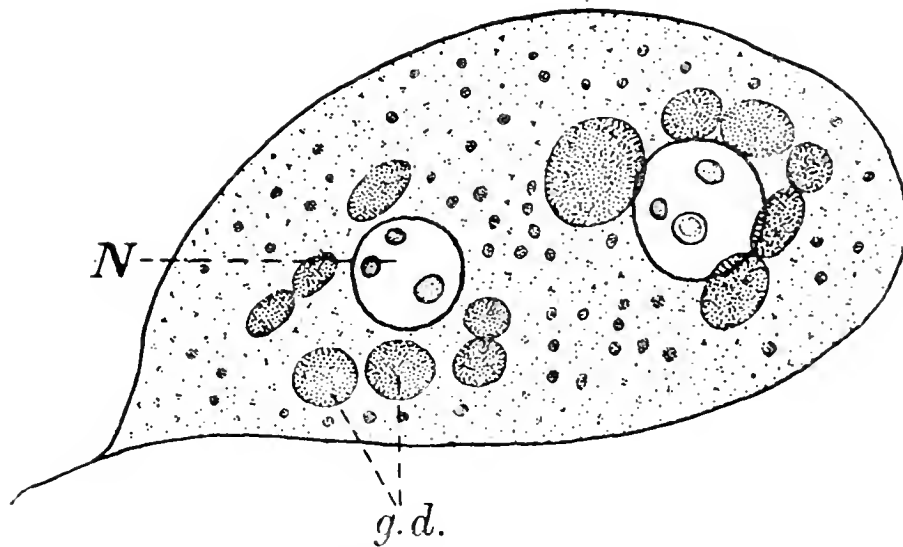


Fig. 2. A living nephrocyte of *P. humanus* slightly compressed, showing two nuclei, *N*, and green droplets, *g.d.*

Disseminated Nephrocytes in Pediculus.

To examine these structures, the living insect should be opened and the fat body removed and spread in a drop of normal salt solution. The specimen is then covered lightly with a cover-glass and examined microscopically. The large polynuclear cells of the fat body, filled with fat droplets, are seen to be connected by much smaller cells. These cells are binucleate and in respect to their protoplasmic structure and inclusions conform to the above described peri-oesophageal nephrocytes, but for their disposition and shape (Fig. 3).

They are more elongated and are more or less fused in a chain-like manner, the terminal nephrocyte being either fused with the fat body or connected therewith by means of a long filament.

The Physiological Function of the Nephrocytes.

The cytological structure of the nephrocytes we have considered above, strongly resembles that of the pericardial cells of other insects as already pointed out by Müller. It is therefore natural to ask if the function of these cells is not similar to that of the pericardial cells. With a view to replying to this question it is necessary to dwell upon what is known regarding the physiological function of pericardial cells.

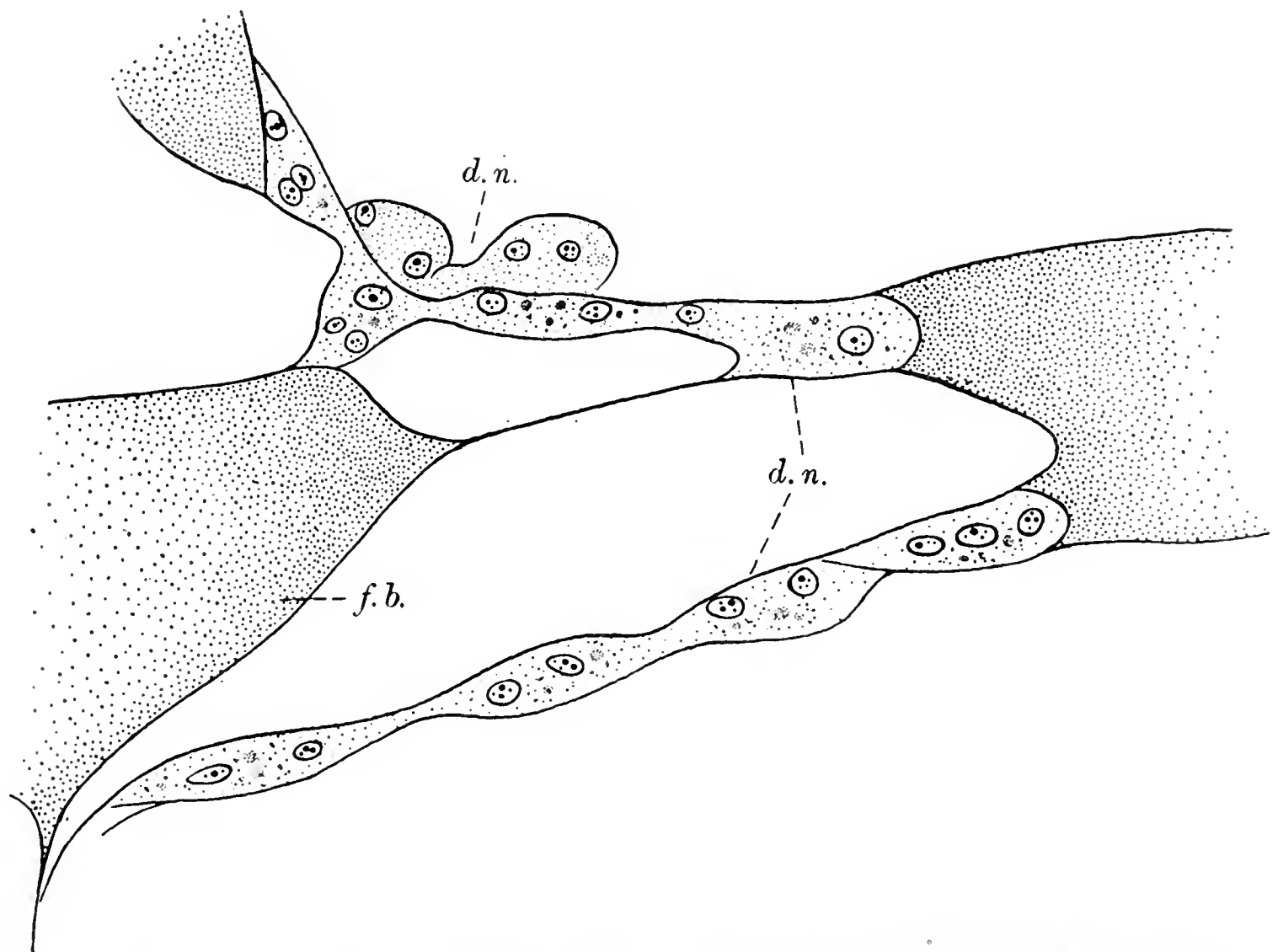


Fig. 3. Disseminated nephrocytes of *P. humanus*, *d.n.*; connected with the fat body, *f.b.*

Kowalevsky (1886) was the first to show that in Dipterous larvae, fed upon artificially coloured food, the pericardial cells become stained more or less intensely. He also showed that the colouring substances he employed, namely cochineal powder and silver salts, become deposited in the protoplasm of the pericardial cells without, however, staining their nuclei.

In 1889, Kowalevsky, moreover, showed that these cells are capable of eliminating certain substances introduced into the body either (*a*) directly by injection into the body cavity, or (*b*) indirectly through the alimentary canal, and, since the cells do not possess an excretory duct, they store throughout their life the substances which they take up.

In his injection experiments Kowalevsky used various substances like ammonia-carmin, indigo-carmin, methylene blue, alizarine, and finally blue litmus on the advice of Metchnikoff. The employment of litmus was of special value since it showed that the pericardial cells possess an acid reaction, for, when the animal is injected with blue litmus the cells turn the absorbed litmus red. Kowalevsky therefore concluded that the pericardial cells constitute excretory accumulatory organs having an acid reaction. It may be noted here that the Malpighian tubes of insects possess a distinctly alkaline reaction.

In 1892, Kowalevsky, whilst confirming his previous observations, found that the pericardial cells are incapable of taking up solid particles, these being taken up by lymphocytes or leucocytes only.

The chief results of Kowalevsky were confirmed by Cuénot (1896) and Metalnikoff (1896). Subsequently Bruntz (1903) in his studies upon excretion in Arthropods, wherein he injected ammonia-carmin and indigo-carmin, was able to show that a great variation exists in the form, grouping and distribution of the excretory cells to which he applied the name "*nephrocytes*." He found these frequently situated at a distance from the heart and lying scattered between the lobes of the fat body. Finally, Keilin (1917) has described the nephrocytes in several species of dipterous larvae, and observed cases (*Lonchae vaginalis*) where the nephrocytes under natural conditions were coloured, according to the age of the larva, either light brown or black by the accumulation within them of excretory substances.

To determine the excretory function of the nephrocytes in *Pediculus*, it was necessary to employ one of two methods that have been used in similar studies upon other insects, the two methods being (1) to feed the insects with coloured fluid, or (2) to inject such coloured matter into their body cavity. Owing to lice being solely blood-sucking insects, we were obliged to carry out our experiments by the second method only. After a few preliminary trials we succeeded perfectly in obtaining satisfactory results, and, since it may prove useful to other investigators, we shall describe our method of performing such experiments.

Method of performing Intra-coelomic Injection upon Pediculus.

The first step is to prepare fine glass pipettes from tubes measuring 1 mm. in diameter and 7–8 cm. in length. One end of the tube is drawn out to a fine but rigid point which is broken off with a forceps under the binocular microscope so that a fine lumen can be discerned.

The best results are obtained with lice maintained in the thermostat at 30° C. for about 16 hours after a meal, replete lice being unsuitable for injection. Holding the louse between the forefinger and thumb of the left hand, the point of the pipette, charged with a small amount of injecting fluid and held in the right hand, is driven into the side of the insect's abdomen, gentle pressure only being exerted. As soon as the point has traversed the external

integument, the insect should be released by the fingers, whilst it remains impaled on the pipette point. The operator now places the free end of the pipette between his lips and blows some of the fluid it contains into the insect's body cavity. As soon as this is accomplished the louse is detached from the pipette by sliding a needle along the glass. The insect, after resting on cloth at room temperature for an hour, is fed and afterwards returned to the thermostat. After 20–24 hours, if the louse has been injected with ammonia-carmines, the nephrocytes of the living specimen are found to be coloured red when viewed by transparency.

We would remark that before reaching conclusions regarding experiments of this character it is necessary to confine one's attention solely to the living insects because more or less generalized coloration may take place in dead or dying lice.

Results of Intra-coelomic Injections of ammonia-carmines in Pediculus.

Of 22 experimental injections of the foregoing description carried out by us on *Pediculus humanus*, 14 gave good results. The failures were due either to the lice being injured through laceration of the gut or through precipitation of the injected carmine having occurred. The successfully treated lice were killed for purposes of examination 3–5 days after injection. One specimen (♀) injected on 4. vi. 1918, which showed coloured nephrocytes after 24 hours, survived until 21. vi, when she was killed. In other words she lived for 16 days, and laid 62 eggs during this period, her nephrocytes remaining coloured throughout. It is evident therefore that the technique when successful does not involve material injury to the louse.

When the lice are examined 24 hours after injection no traces of ammonia-carmines are visible in the perivisceral fluid, on the other hand all the nephrocytes are found to be coloured red. When the lice are immersed in water and slightly compressed between slide and cover-glass, being viewed dorsally with the binocular microscope, the exact distribution of the nephrocytes can be clearly discerned. As shown in Fig. 4, as a rule, two red peri-oesophageal agglomerations of nephrocytes are visible, at times the masses are fused in the form of an irregular crescent.

Apart from the foregoing, small groups of red cells are seen irregularly distributed beneath the dorsal surface. The distribution of these cells varies in different individuals and in one and the same individual according to its state of repletion, or if the specimen is a female, according to the state of development of the ova. These cells or groups of cells, when dissections are made, are seen to be united to the fat body as shown in Fig. 5, and they correspond in every way to the cells we have already described as dorsal or disseminated nephrocytes. When examined under a high power, they exhibit the two characteristic nuclei which are hidden in the illustration through the accumulation of carmine granules within the cells.

In two cases the nephrocytes were so charged with granules of carmine that they began to break up thereby liberating carmine granules which were promptly taken up by the leucocytes in the perivisceral cavity. Soon after-

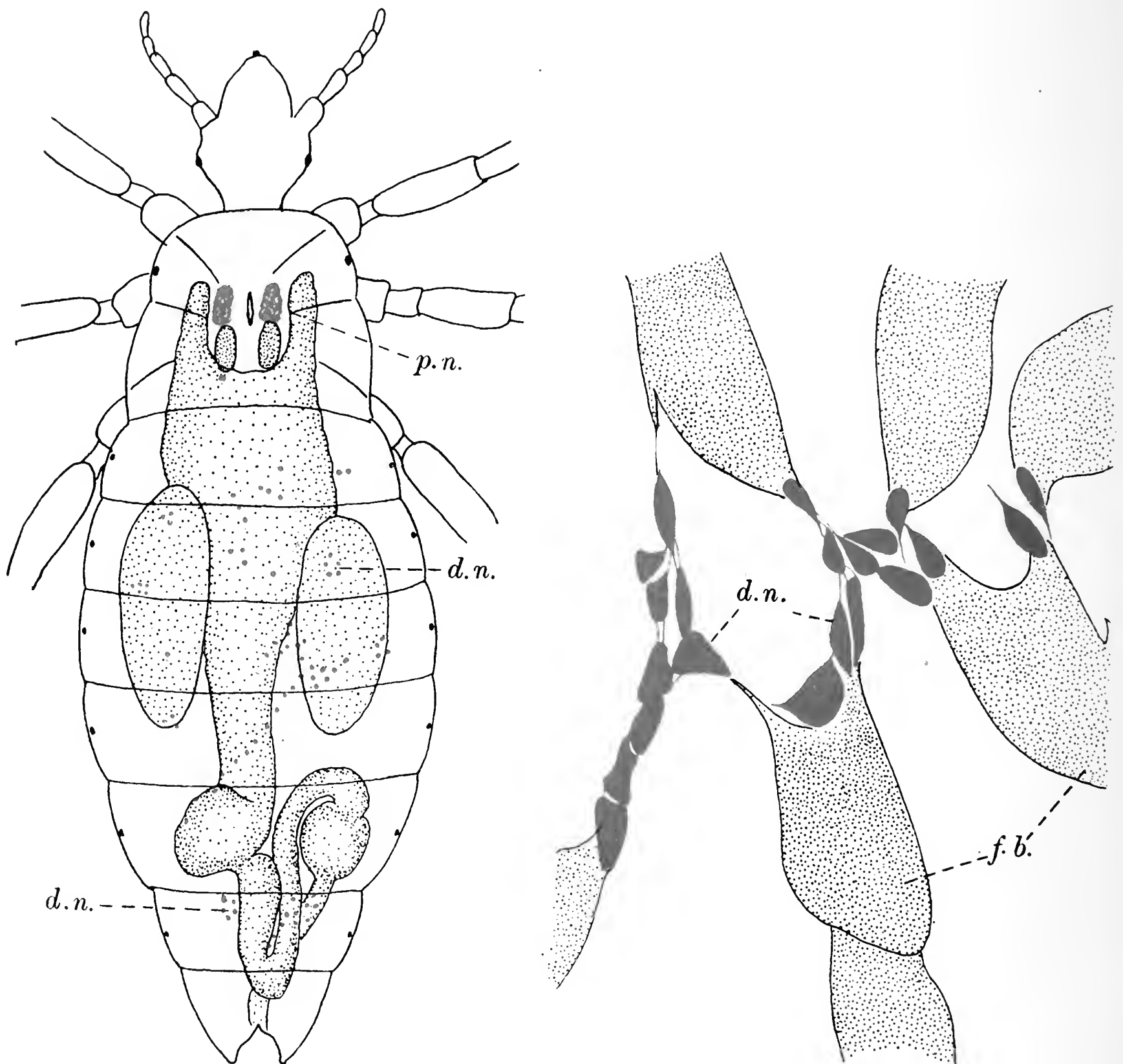


Fig. 4. *Pediculus humanus* ♀, 24 hours after being injected with ammonia-carmin, showing the nephrocytes filled with carmine: *d.n.*, disseminated dorsal nephrocytes; *p.n.*, peri-oesophageal or ventral nephrocytes.

Fig. 5. Dorsal or disseminated nephrocytes linked with the fat body cells, extracted from *P. humanus* after injection with ammonia-carmin: *d.n.*, dorsal nephrocytes; *f.b.*, fat body.

wards, in all parts of the body, even in the head and legs, were found isolated or aggregated leucocytes charged with carmine granules, the appearance being identical with that following the injection of a suspension of powdered carmine into the body cavity of an insect.

Nephrocytes of Mallophaga.

Essentially similar to the peri-oesophageal nephrocytes of *Pediculus* are certain groups of cells described by various authors in Mallophaga. Thus Kramer (1869, p. 455) working with *Lipeurus jejunos*, found, connected with the smaller pair of salivary glands, a peculiar complex of about 14 large cells grouped in two rows, the cells "mostly containing two or four nuclei." These cells showed no efferent duct, but, "because of their constant seat of attachment at the end of the gland opposite to where its duct issues," they were regarded by Kramer as necessarily bearing some relation to the salivary secretion.

Grosse (1885, p. 549) found similar cells to those described by Kramer in *Nirmus*, *Trichodectes*, *Lipeurus versicolor* and *L. heterographus*. The cells were not, however, confined to the crop region, but also occurred in other parts of the body in groups of 2-6-8 upon the fat body. Grosse argued that since the cells are so generally distributed in the insect's body their salivary function, as supposed by Kramer, is doubtful.

Snodgrass (1899, pp. 167-8) on the other hand, figured and supposed that he discerned an efferent duct leading from similar cells in *Trichodectes geomydis*, the duct opening at the junction of the crop and oesophagus. In *Eurymetopus*, *Docophorus* and *Gonoides*, the cells are more numerous than in *Trichodectes* and their "ducts" are difficult to see. According to Snodgrass each "cell possesses two nuclear-like bodies (one of these may be a hollow space into which the duct opens, such spaces being present in salivary cells of insects), and they are all closely pressed together so that they assume polygonal shapes." The observations of Snodgrass are not in accord with those of Kramer and Grosse, and they appear very doubtful because the nephrocytes are usually tangled up with tracheae, nerves, muscle fibres and real salivary ducts, any of which Snodgrass may have taken for a "duct" leading from the nephrocytes.

Nephrocytes of Pediculus and Diptera compared.

There remains to be considered the distribution of the nephrocytes observed in dipterous larvae since they are comparable with those in *Pediculus*.

According to Weismann, Kowalevsky, Bruntz and Keilin, the nephrocytes in dipterous larvae are distributed in two distinct groups: (a) One group occurs in the form of two parallel dorsal cell-chains applied to the sides of the heart; these are the pericardial cells in the strict sense. (b) The second group of nephrocytes forms a festoon-like chain ("girlandenförmiger Zellenstrang" of Weismann) that is always found suspended ventrally between the two salivary glands (see Keilin, 1917, pl. XII, fig. 46 n.v.). It is highly probable that the two groups of nephrocytes in dipterous larvae correspond respectively to the disseminated nephrocytes (dorsal) and peri-oesophageal nephrocytes of *Pediculus*, especially since according to Sikora (p. 58) in *Haematopinus eurysternus* the dorsal nephrocytes occupy a pericardial position.

Fulmek (1909, pp. 56-58, pls. I, II), moreover, has shown that in Mallophaga the pericardial cells are closely aggregated at the sides of the heart.

SUMMARY.

In *Pediculus humanus* are found two groups of excretory-accumulatory cells known as nephrocytes.

The one group, the peri-oesophageal, lies ventrally and consists of large cells aggregated usually in two masses about the oesophagus anterior to the reniform salivary glands.

The second group lies dorsally and consists of disseminated cell aggregates linked with the fat body.

The typical nephrocyte is a binucleate cell with granular protoplasm containing greenish droplets of varying size. The excretory function of the nephrocytes is demonstrated by intra-coelomic injection of ammonia-carmin. The latter, 24 hours after injection, is taken up by the nephrocytes which become red and filled with the carmine granules. These granules remain in the protoplasm of the nephrocytes throughout the life of the insect.

Similar cells exist in Mallophaga and have been wrongly described by some authors as salivary glands.

The two groups of nephrocytes, described by us in *Pediculus*, occur in other insects, but the dorsal group in the latter forms usually two chains of cells (known as pericardial cells) lying on either side of the heart.

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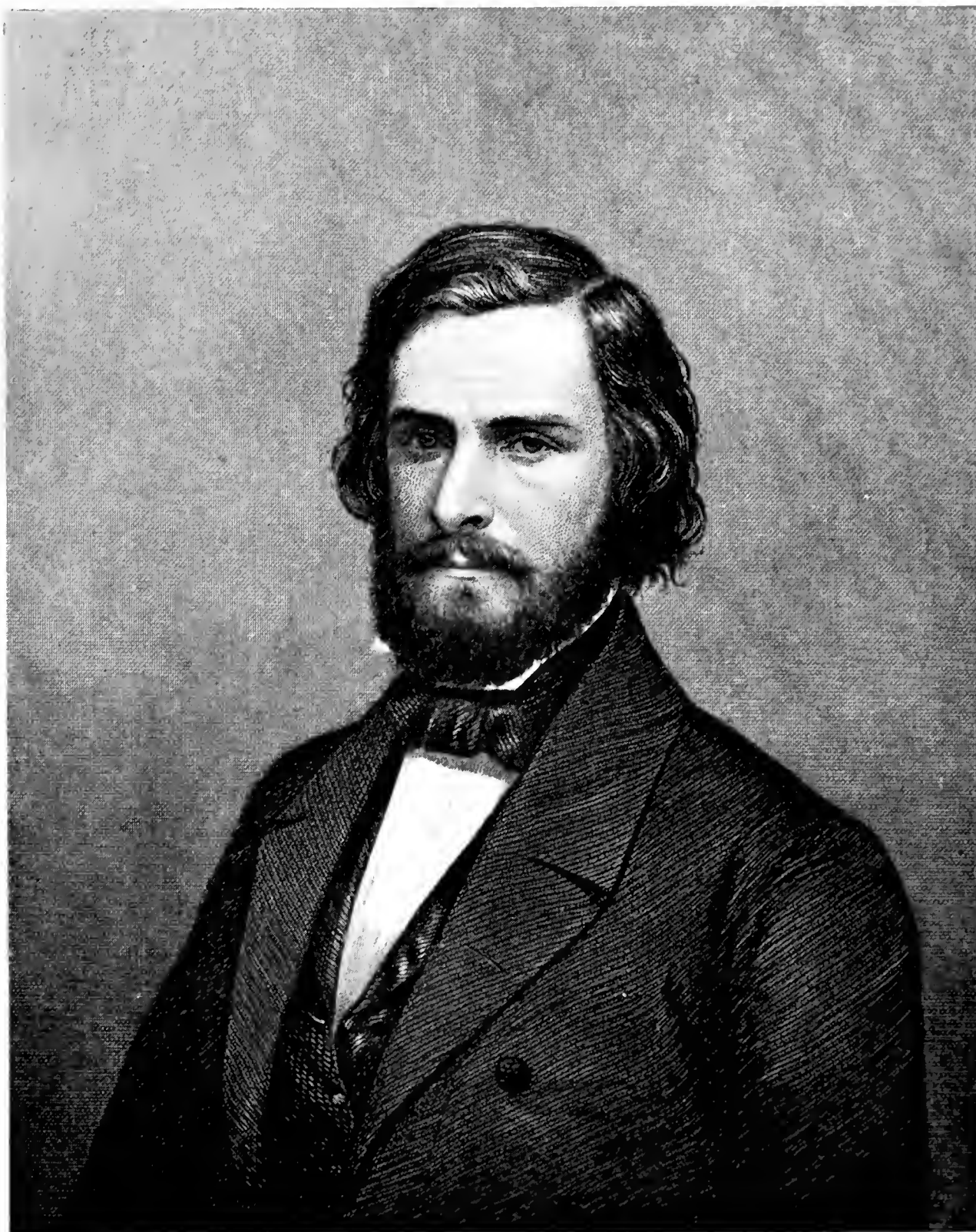
KARL ASMUND RUDOLPHI

1771—1832



JOHANNES MÜLLER

1801—1858



JOSEPH LEIDY

1823—1891

ON A NEW CESTODE FROM THE POUCHED RAT,
CRICETOMYS GAMBIANUM.

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*(From the Research Laboratory in Agricultural Zoology,
University of Birmingham.)*

(With Plate IX.)

THE material of the species to be described was found in a small collection of parasites from Zanzibar, placed at my disposal by Professor G. H. F. Nuttall, F.R.S., to whom I wish to express my thanks. As only a few cestodes have hitherto been recorded from East Africa and its helminth fauna is therefore largely unknown, a list of the forms identified is given at the end of the paper. I desire to acknowledge my indebtedness to the Birmingham Natural History and Philosophical Society for a grant towards the expense of preparing the plates, both in this and the preceding paper (*Parasitology*, XIII. No. 1, March 1921, p. 1).

The specimens of the new species were obtained from the duodenum of the pouched rat (*Cricetomys gambianum*), and consist of five strobili, two with scoleces, and numerous fragments. The scolex (Pl. IX, fig. 3) is 0.5 mm. long \times 0.66–0.75 mm. diameter, is furnished with a small apical depression devoid of any muscular structure and with four suckers, two dorsal and two ventral. These lie in pockets inside the scolex, opening to the exterior by a small orifice. The musculature of the sucker does not completely fill the pocket but leaves vacant near the opening a small non-muscular circle, sharply crinkled round the edges. Considerable difference of opinion exists upon the position of the suckers in this genus. According to Klapotcz (1906, 139) in *I. gondokorensis* they are to be found inside the scolex at the bottom of long funnel-shaped pockets: this is true of *I. hyracis*, *I. interpositus* and *I. setti* according to von Janicki (1910, 394) and is cautiously confirmed by Bischoff (1913, 248) for his own species; "Auch alle von mir untersuchten Arten von *Inermicapsifer* wiesen teils mehr, teils weniger deutlich diese typischen Saugnapftaschen auf." On the other hand Beddard (1912, 578) states: "Nor could I detect anything peculiar in the structure of the suckers, which, according to Janicki and others, are remarkable for a funnel-like ingrowth leading to the actual sucker, which thus lies at the bottom of a depression. It is true that

in the present species, as in many tapeworms of the group *Tetracotylea* which I have examined, the sucker does not lie externally on the scolex, but is covered by a layer of body-wall which is only interrupted at the orifice of the sucker. The free edge of this, when depressed towards the interior of the sucker, is doubtless funnel-shaped and would give rise to the appearances represented by Janicki. Perhaps, however, there is some divergence from the normal condition of the suckers in the members of the genus referred to by him, which I certainly have not found in the species with which I am at present concerned." The suckers figured by von Linstow (1906, Pl. XIII, fig. 9) for *Z. remota* and Pagenstecher (1878, Pl. X, fig. 2) for *I. criticus* are obviously on the surface of the scolex in the usual Cyclophyllidean position. In *Z. muricola*, according to Baylis (1915, 41), they occupy an intermediate position, "they are sunk somewhat deeply in the substances of the head, and their orifices are flush with the surface." My own observations confirm the statement of Beddard just quoted. The suckers are distinctly under the surface of the scolex but not at the bottom of the funnels and they thus present a very different appearance to the usual Cyclophyllidean sucker with a rim of musculature on the external surface of the head. The greatest diameter of the suckers observed was 0.2 mm. Behind them the scolex bulged to a considerable extent, its posterior limit being twice the diameter of the long unsegmented neck which follows it. The proglottides are broader than long for the greater part of the strobili, the more terminal ones being approximately square.

The musculature of each proglottis (Pl. IX, figs. 5-7) is exceedingly weak, consisting of only two layers, one transverse and one longitudinal. The latter is composed of a number of isolated fibres—only occasionally do two or three of them become aggregated together into a bundle—scattered irregularly throughout the cortical parenchyma. Internally to them lie several strands of transverse muscles forming a band enclosing the genital organs and medullary parenchyma.

The excretory system consists of the usual four longitudinal vessels, two on each side of the proglottis—these being arranged laterally to each other instead of dorsally and ventrally. The innermost is large and thin-walled and communicates with its fellow of the opposite side by a large transverse commissure at the posterior margin of the proglottis. It also gives off here an outer vessel the branches of which, running anteriorly, anastomose with each other and with the parent stem, forming a small excretory plexus. The outer longitudinal vessel is small and thick-walled, gives off no lateral branches, and does not communicate with that on the opposite side by any transverse commissures.

The genital pore is unilateral, nearly at the anterior quarter of the proglottis margin (Pl. IX, fig. 4). A small and narrow genital cloaca is present and into it open anteriorly and posteriorly the cirrus-sac and vagina respectively. The genital ducts pass between the excretory vessels and—assuming the larger excretory vessel to correspond to the ventral vessel usually present and the smaller to the dorsal—dorsal to the nerve.

The cirrus-sac is small and pear-shaped, the greatest diameter being internally, 0.14–0.16 mm. long \times 0.04 mm. dia., and does not reach the nerve. The cirrus is unarmed and opens into a small straight vesicula seminalis which fills the remainder of the sac. Outside the cirrus-sac the vas deferens forms a bundle of loose coils extending as far as the inner longitudinal excretory vessel, there breaking up into the vasa efferentia. The testes are numerous, 120–130 in each proglottis, and form two groups, separated only for a small space by the female organs. On the aporal side they number 106—the number in the proglottides counted was remarkably constant—and are arranged in the form of a wedge with the longer axis lying transversely across the strobilus and the testes more numerous laterally than medianly. They extend as far anteriorly as the margin of the preceding proglottis. Half-way across they diminish to two rows along the posterior border and to a single row at their junction with the female organs. The poral group consists of from 15–20 testes arranged in a double row along the posterior margin and not extending further anteriorly than the vagina. Like the aporal group, these narrow to a single row on reaching the female organs. The two groups are separated only by the diameter of the yolk-gland, appearing under a low magnification to be a continuous row along the posterior margin, the yolk-gland apparently being the connecting link. Laterally both groups extend beyond the two excretory vessels, but do not pass the nerve. Dorso-ventrally the testes lie in a single layer, but in a few places this may be doubled.

The female glands lie porally, a quarter of the distance across the proglottis, internally to both poral excretory vessels. The ovary lies on the ventral surface immediately median to the inner excretory vessel, half-way between the anterior and posterior margins. It is small and straggling, the lobes being long and slender and only slightly connected with one another. Posteriorly and aporally to it is the yolk-gland, also lobed but with blunter processes. The distal portion of the vagina is thick-walled and surrounded with gland cells. On crossing the lateral nerve, this portion communicates by an exceedingly fine duct with a large thin-walled receptaculum seminalis full of spermatozoa, constricted in places, and extending almost to the ovary. Dorsal to the latter is the shell-gland. A definite but ephemeral uterus is developed. The oviduct, after passing through the shell-gland, runs anteriorly and becomes a thick-walled glandular tube with an exceedingly narrow lumen. On arriving dorsally to the receptaculum seminis the lumen increases, the walls become studded with nuclei and the duct, curving internally, opens into the uterus. This latter is a thin-walled sac with a narrow dorso-ventral diameter occupying only one-fifth of the proglottis but extending laterally beyond both excretory vessels and reaching the nerve. In it the eggs are arranged in a single layer: occasionally one or two become pushed one on top of the other but there is never any outgrowth as figured by Janicki (1910, Pl. XII, fig. 13) for *Inermicapsifer hyracis* (Pall.). The degeneration of the uterus and the formation of the egg-capsules agree very well with the account of Janicki just

mentioned. There is a tendency for the eggs in the uterus to become isolated each in separate cavities, and as the proglottis matures this process continues until all the eggs lie in a single dorso-ventral row, each in its own egg-capsule formed from the walls of the disappearing uterus (Pl. IX, fig. 5). These capsules become aggregated together in groups of 9–11 and surrounded by a fibrous, rather spongy modification of the body parenchyma; this forms a distinct sheath round each group and also penetrates in between the individual capsules. Each group of eggs thus becomes completely separated from its neighbours (Pl. IX, fig. 6). In a transverse section there are approximately 16 of these capsules, extending on both sides past the excretory vessels and nearly reaching the nerve: dorso-ventrally they occupy nearly all the space between the two opposite layers of transverse muscle.

Between these capsules there is still left a certain amount of medullary parenchyma, but as growth proceeds this completely disappears, the egg-capsules, becoming polygonal by compression, occupy all the space bounded by the transverse muscles and form a double dorso-ventral layer instead of a single one (Pl. IX, fig. 7). Each capsule has now a definite delimiting membrane externally and consists of two portions: an outer fibrous spongy mass, and an inner granular non-nucleated matrix in which are imbedded the eggs. I could not find any traces of the second layer figured by Baylis (1915, fig. 6). In each proglottis, now approximately square, are 40–50 egg-capsules each containing 9–11 eggs and filling all the space between the two longitudinal nerves in one direction and between the anterior and posterior margins of the proglottis in the other.

The possession of an unarmed scolex and the absence of a persistent uterus place this worm in the sub-family *Linstowinae* Fuhrmann 1907. This includes up to the present six genera, two of which, *Linstowia* Zschokke 1898 and *Oochoristica* Lühe 1898, are definitely separated from the form under discussion by the position of the female organs in the median portion of the proglottis. The third genus, *Hyracotaenia* Beddard 1912, by the absence of a persistent uterus (a character which should remove the genus from this sub-family) can therefore be dismissed. The other three genera, *Inermicapsifer* v. Janicki 1910, *Zschokkeella* Fuhrmann 1902 and *Thysanotaenia* Beddard 1911 are closely allied and, up to the present, insufficiently separated from each other.

The only characters distinguishing *Thysanotaenia* appear to be the poor development of the excretory system, the more median position of the female organs and the larger size of the cirrus-sac. The variability of the excretory system is often very great in allied species; in *Oochoristica crassiceps* Baylis (1920, p. 293) found no excretory plexus nor any transverse commissures; on the other hand in *O. wagneri* v. Janicki the excretory system is well developed. Rudin (1917, p. 317) states: "Ganz ausserordentlich sind die verschiedenen Formen der Ausbildung, die das Excretionssystem, nicht nur innerhalb der Genera *Ophiotaenia* und *Acanthotaenia*, sondern im allgemeinen innerhalb der

Familie der Ichthyotaenien, aufweist," proceeding then to elaborate the differences between the species of the same genus. The position of the female glands in the species of a genus is not constant. In the genus *Davainea* Blanchard 1891 they customarily lie on the median axis of the strobilus, but in *D. leptotrachela* Hungerbühler 1910 they are poral in position, nearly touching the longitudinal excretory vessels, and again in *D. tetragona* (Molin 1858) and *D. echinobothrida* (Méglin 1880) are distinctly poral. With regard to the size of the cirrus-sac, in his account of the type species, *T. lemuris*, Beddard (1911 a) gives neither measurements nor a statement of its extent, merely stating (1912, p. 607) "Cirrus-sac rather large," a vague statement unsuitable for use as a generic character. Neither do the three characters taken in combination assume sufficient importance to be of generic rank. From Beddard's description therefore, unless subsequently further distinctive characters be forthcoming, I would suggest the placing of *T. lemuris* in the genus *Zschokkeella*,—the egg-capsules being formed altogether independently of the uterus (1911, p. 1000)—and the suppression of the genus *Thysanotaenia*.

For the identification of the two remaining genera Beddard (1912, p. 607) has provided a somewhat unsatisfactory key. He places *I. hyracis* (Pall.)¹ in the genus *Zschokkeella* and then distinguishes between the two genera by crediting *Inermicapsifer* with posterior genital pores, two groups of testes entirely separated from each other, a vas deferens together with a seminal network, and the absence of a well-developed uterus, and *Zschokkeella* with median genital pores, a continuous row of testes, the absence of a vesicula seminalis (and presumably of a seminal network?) and the presence of a well-developed uterus. With regard to the first character, the disposition of the testes, there is every variation between the type of *I. setti* v. Janicki 1910 with the testes in two clearly separated groups, through *Z. gambianum* Beddard 1911 (1912, p. 582), where three testes form a connecting link between the two groups to *I. hyracis* (Pall.) where they are arranged in a continuous band along the posterior border of the segment, and *I. paronae* Bischoff 1912, where they are equally distributed throughout the whole proglottis, extending anteriorly to the genital ducts. For *Zschokkeella muricola* Baylis (1915, p. 47) states: "The testes are divisible into two groups; but they so nearly form a complete series across the segment (except where interrupted by the female organs) that it is difficult to say whether their arrangement is more like that seen in *Inermicapsifer* or that in *Zschokkeella*. In this respect the species seems to be intermediate between the two genera." A character so liable to variation as this cannot be considered adequate for the discrimination of genera. The further distinction drawn by means of the vesicula seminalis is inaccurate. Fuhrmann (1902, p. 139) for *Z. linstowi*, the type species

¹ *Taenia hyracis* Pallas 1767 was selected by von Janicki (1910) for the type species of his genus *Inermicapsifer*. If this species be removed to the genus *Zschokkeella* (Fuhrmann 1901), the name *Inermicapsifer* falls into synonymy with *Zschokkeella* and Beddard's first group, with testes in two distinct groups, should be renamed.

where, according to Beddard's table, it should be absent, states: "Am inner Ende der schwach muskulösen Penistasche liegt eine kleinere Vesicula seminalis." On the other hand, Baylis (1915, p. 43) found it absent in *Z. muricola* while, with the exception of *I. capensis* Beddard 1912 and the new species just described, it is absent or, being unmentioned, presumably absent in all the nominal species of *Inermicapsifer*, whether with a continuous band of testes and therefore referable to *Zschokkeella*, or with two groups and therefore referable to *Inermicapsifer*. The position of the genital pore, the third character, is anterior in *I. parvulus* Bischoff 1912, and posterior in *I. setti* v. Janicki 1910—both with two separate groups of testes—and anterior in *Z. remota* v. Linstow 1906 and posterior in *I. parona* Bischoff 1912—both with a continuous band of testes. The last character in the key, uterus well or little developed, is a comparative one and therefore suffers from vagueness. In *I. hyracis* the uterus is stated Janicki (1910, p. 381) to extend between the excretory vessels as a broad sack with outgrowths, disappearing to form the walls of the primary egg-capsules: in *I. capensis* the oviduct is stated (Beddard 1912, 586), to be "quite short and ends more or less abruptly in a strand of condensed parenchymal tissue." In *Z. gambianum* (Beddard 1911, p. 659) the uterus is "not much more than a transversely running tube extending nearly right across the proglottis in which the eggs occur, but with which the uterus never appears to be stuffed. I could find no outgrowths of this centrally-placed uterus, and there was certainly nothing in the nature of a reticular formation of its cavity." Other investigators are content merely to state, "Der Uterus, der sich auf jüngerem Stadium unregelmässig zwischen die Hoden einschiebt, löst sich etwa im letzten Viertel der Strobila sehr rasch in Eikapseln auf" (Bischoff 1913, p. 238): "The uterus persists through about 28 segments, but after a short time appears to break down altogether, and the ova are seen to be scattered among the parenchyma" (Baylis 1915, p. 44): and "Der Uterus bildet anfangs ein median verlaufendes, leicht gewelltes Rohr, das beiderseits bis an die Längsnerven reicht. Seine Wandung verscheint bald und werden die Eier ins Parenchyme gestossen" (Fuhrmann 1902, 140). *I. capensis* is the only species which can thus definitely be placed in the genus *Inermicapsifer*, the remaining 17 species falling into the genus *Zschokkeella* which then becomes a rather heterogeneous assemblage, for the testes may be continuous (*I. hyracis*) or in two groups (*I. setti*), a vesicula seminalis may be present (*Z. linstowi*) or absent (*Z. muricola*), and the genital pore posterior (*I. paronae*), or median (*I. interpositus* v. Janicki 1910), or anterior (*Z. muricola*) and there may be almost any permutation and combination of these characters. From the above facts it may be seen that it is impossible to use Beddard's definition of the two genera unless it is prepared to reserve the genus *Inermicapsifer* for *I. capensis* alone and make *Zschokkeella* a dumping ground for all the other species.

Von Janicki (1910, p. 394) distinguishes his genus from *Zschokkeella* by the following points: eggs in parenchymatous capsules and not merely in "Binde-

gewebe," suckers sunk in pockets, not on the surface of the scolex; greater extension internally of the ventral excretory system, the greater development of medullary parenchyma and of parenchymatous muscles; the greater length of the terminal proglottides; the restriction of the testes to the more posterior portion of the proglottis; and the position of the female organs median to the excretory system. I am in entire agreement with Beddard's criticism of this (1912, p. 606): the points mentioned are either trivial or not in accordance with the facts.

There only remains the question of the formation of the egg-capsules. For *I. hyracis* (p. 381), *I. interpositus* (p. 386) and *I. setti* (p. 388) v. Janicki asserts that the eggs are first enclosed singly in capsules formed from the uterine walls and only secondarily in capsules formed of modified medullary parenchyma; after this, the primary capsule wall disintegrates—a process similar to that described by him for *D. polycalceola* (1902, 258). This account is borne out by Bischoff for *I. paronae* (1913, 238) and *I. lopas* (p. 244) and confirmed by the behaviour of the uterus in the species described at the commencement of the paper. On the other hand Beddard (1912, 587) asserts that in *I. capensis* the uterus is never developed as a definite tube with a lumen and that therefore the egg-capsules can have no connection with it, and for *Z. gambianum* states (1911, 659): "Gradually the cavity of the uterus appeared, as it were, to dry up and the eggs were found—to continue the simile—stranded in the tissue of the body." Baylis again for *Z. muricola* states (1915, 45): "There seems to be no ground for supposing that the capsules in which the eggs are enclosed, whether they are to be regarded as 'para-uterine organs' or not, are derived from the uterus." In his definition of *Zschokkeella* Fuhrmann states (1908, 40): "Uterus löst sich in Eikapseln auf," but on turning to his description of the type species (1901, 761) he there says: "Der Uterus löst sich auf, wie dies für das Genus *Linstowia* charakteristisch ist, und kommen die Eier ins Parenchyme eingebettet zu liegen"; and (1902, 140): "Uterus verliert seine Wandung bald und die Eier treten ins Parenchyme über." (See also the quotation already given.) From these latter descriptions it does not appear that Fuhrmann wished to imply that the eggs are enclosed in capsules formed directly from the uterine walls, but that, on the contrary, they are deposited in the parenchyma and the capsules subsequently form around them, a process similar to that described by Beddard for *I. capensis* and *Z. gambianum* and in direct contradiction to Janicki's account of *I. hyracis*, *I. interpositus* and *I. setti*. This point seems to allow of a distinction being drawn between the two genera and I would suggest that the generic diagnoses be altered into "Eggs at first enclosed singly in capsules with walls derived from the uterus and only secondarily in capsules with walls of modified parenchyma" for *Inermicapsifer* and "Eggs enclosed singly, or in groups, in capsules in the formation of which the uterus has no part" for *Zschokkeella*. This would place definitely in *Inermicapsifer* the species *I. hyracis* (Pallas 1767), *I. setti* v. Janicki 1910, *I. interpositus* v. Janicki 1910, *I. lopas* Bischoff 1912

Cricetomys gambianum

	Suckers	Genital pore	Testes	Vesicula semi-nalis	Ovary	Uterus	Formation of egg capsules	Number of egg capsules	Number of eggs per egg capsule	Host
<i>Inermicapsifer</i>										
<i>I. abyssinicus</i> Bischoff 1912	As in <i>I. interpositus</i>	In centre of proglottis margin	50-60, as in <i>I. interpositus</i>	—	—	—	—	24-26	8-10	<i>Procapra</i> sp.
<i>I. apospamion</i> Bischoff 1912	At bottom of pockets	In centre of proglottis margin	In two unequal clearly separated groups	—	Immediately median to poral (ventral?) excretory vessel	—	—	18-24	7	<i>Procapra</i> sp.
<i>I. capensis</i> Beldard 1912	Beneath surface of scolex	In posterior portion of proglottis margin	In two unequal clearly separated groups	Present	Median to both poral excretory vessels	Represented by condensed branched parenchymal tissue	Uterus has no part in their formation	—	—	<i>Procapra capensis</i> Schreber
<i>I. criticus</i> (Pagenstecher 1878)	On surface of scolex	In centre of proglottis margin	In a continuous posterior band?	—	—	—	—	180	—	<i>Procapra capensis</i> Schreber
<i>I. gondokorensis</i> Klapotocz 1906	At bottom of pockets	—	—	—	—	—	—	—	—	<i>Procapra slatini</i> Sassi
<i>I. hyracis</i> (Pallas 1767)	At bottom of pockets	In centre of proglottis margin	100-110, in a continuous posterior band	Absent	Dorsal to poral ventral excretory vessel	Well-developed sac containing eggs	Uterus forms primary capsule	80-100	4-5	<i>Procapra</i> sp.
<i>I. interpositus</i> v. Janicki 1910	At bottom of pockets	In centre of proglottis margin	80, in two continuous posterior rows	—	—	As in <i>I. hyracis</i>	As in <i>I. hyracis</i>	75	5-7	<i>Procapra</i> sp.
<i>I. lopas</i> Bischoff 1912	At bottom of pockets	In posterior portion of proglottis margin	60, in two clearly separated groups	—	Immediately median to poral (ventral?) excretory vessel	—	As in <i>I. hyracis</i>	12-15	12-16	<i>Procapra</i> sp.
<i>I. pagenstecheri</i> (Setti 1897)	On surface of scolex	In posterior portion of proglottis margin	Scattered throughout the entire proglottis	—	Poral	—	—	70-80	8-10	<i>Procapra</i> sp.
<i>I. paronae</i> Bischoff 1912	At bottom of pockets	In posterior portion of proglottis margin	Scattered throughout the entire proglottis	—	Median to poral ventral excretory vessel	As in <i>I. hyracis</i>	As in <i>I. hyracis</i>	30-50	6	<i>Procapra</i> sp.

<i>I. parvulus</i> Bischoff 1912	At bottom of pockets	In anterior portion of proglottis margin	20, in two unequal clearly separated groups	—	—	—	—	18-24	5-7	<i>Procavia</i> sp.
<i>I. prionodes</i> Bischoff 1912	At bottom of pockets	In posterior portion of proglottis margin	40, in a small layer a little posterior to the centre of the proglottis	—	Median to poral (ventral?) excretory vessel	—	—	26-28	5	<i>Procavia</i> sp.
<i>I. setti</i> v. Janicki 1910	At bottom of pockets	In posterior portion of proglottis margin	55, in two clearly separated groups	—	—	As in <i>I. hyracis</i>	As in <i>I. hyracis</i>	20	15	<i>Procavia</i> sp.
<i>I. zanzibarensis</i> n.sp.	Beneath surface of scolex	In anterior portion of proglottis margin	120-130, in two posterior indistinctly separated groups	Present	Median to poral ventral excretory vessel	Poorly developed	As in <i>I. hyracis</i>	40-50	10	<i>Cricetomys gambianum</i> Eth.
<i>I. sp. v. Janicki</i> 1910	Beneath surface of scolex	—	—	—	—	—	—	—	—	<i>Procavia syriaca</i> Ehrbg.
<i>Zschokkeella</i>										
<i>Z. gambianum</i> Beddard 1911	Beneath surface of scolex	In centre of proglottis margin	In two posterior clearly separated groups	Absent	Double each side the poral ventral excretory vessel	Transverse tube devoid of eggs	Uterus has no part in their formation	—	—	<i>Cricetomys gambianum</i> Eth.
<i>Z. linstowi</i> (Parona 1885)	On surface of scolex	In anterior portion of proglottis margin	140, in two lateral groups united by a posterior row	Present	Between the two poral excretory vessels	Transverse tube	—	—	—	<i>Namida pilorhyncha</i> Licht.
<i>Z. muricola</i> Baylis 1920	Beneath surface of scolex	In anterior portion of proglottis margin	In two posterior practically continuous groups	Absent	—	Oblong sac	Uterus has no part in their formation	—	20	<i>Epimys rattus</i>
<i>Z. remota</i> v. Linstow 1905	On surface of scolex	In anterior portion of proglottis margin, slightly ventral	In a continuous band?	—	Median	—	—	—	—	<i>Cercopithecus pyrrhonotus</i> Ehrenb.
<i>Thysanotaenia</i>										
<i>T. lemuris</i> Beddard 1911	Beneath surface of scolex	In anterior portion of proglottis margin	In a continuous posterior band	Present	Anterior, slightly poral	Absent	—	—	3-4	<i>Lemur macaco</i>

and *I. paronae* Bischoff 1912, and in *Zschokkeella* the species *Z. linstowi* (Parona 1900), *Z. muricola* Baylis 1915, *Z. gambianum* (Beddard 1911), *I. capensis* Beddard 1912, and *Th. lemuris* Beddard 1911. In the remaining species the behaviour of the uterus has not been described and therefore they cannot be ascribed to either genus. A difference in the formation of the egg-capsules, as Beddard has pointed out, is hardly sufficient to distinguish two genera but, failing this, *Inermicapsifer* must fall into synonymy with *Zschokkeella* as no other character exists by which they can be distinguished one from the other. Until, therefore, a more detailed account of the behaviour of the uterus throughout the group be published it seems best to leave the two genera separated by this difference.

In accordance with this decision the new species described at the commencement of the paper (p. 193) belongs to the genus *Inermicapsifer* and by means of the table on pp. 200–201 can be distinguished from the other species. I propose for it therefore the name *Inermicapsifer zanzibarensis* n.sp.

Dipylidium oerley von Rátz, 1900.

The material of this species from the cat consists of three strobili and a few isolated proglottides. The greatest length observed was 40 mm. and the greatest breadth 1 mm. A scolex was not present.

The musculature of the mature proglottides (Pl. IX, fig. 1) in a transverse section consists of three longitudinal muscle bands, each bounded internally by transverse muscles. The innermost longitudinal muscle layer is composed of numerous small bundles, clearly separated one from the other, and formed by the aggregation of small fibres into groups of from 2 to 5: this layer, while distinct in the more posterior portion of the strobilus, becomes less marked anteriorly, until in proglottides with feebly developed male organs it entirely disappears. The layer external to it is wider and more continuous, the bundles are larger and formed of more fibres (approximately 20) and, together with the third layer, it is persistent throughout the proglottis. The outermost longitudinal muscle band is composed of numerous muscle fibres, aggregated into bundles of 5–8 internally and continuing as isolated strands nearly to the sub-cuticula. The transverse muscle layers are very irregular and vary greatly in appearance, occasionally disappearing altogether.

The excretory system in mature proglottides consists of a single large longitudinal vessel on each side, the two vessels communicating by the usual transverse commissure at the posterior end of each segment.

The genital pore is usually situated in a conspicuous depression in the anterior half of the proglottis margin; occasionally the depression is partially evaginated, becoming a small protuberance. The genital ducts pass dorsal to both excretory vessels and to the nerve. The cirrus-sac is 0.21 mm. long \times 0.08 mm. diameter and extends just past the excretory vessels. Its musculature is very feeble and special retractor muscles are absent. The cirrus is

coiled and strengthened by annular thickenings similar to those in the protoxylem of Dicotyledons. When the cirrus lies inside the sac, these thickenings are on the exterior of its walls and project into the cavity of the sac. The space between the coils of the vas deferens is filled with deeply staining cellular tissue. The testes are over 100 and fill the whole proglottis except for the female organs and the short strip occupied by the genital ducts; this latter in stained specimens stands out as a prominent clear space stretching completely across the proglottis.

The eggs in mature segments lie in egg-sacs extending laterally past the excretory vessels. Each sac contains 8–14 eggs. In all other respects the form seen agreed with Hall's description (1920, 63).

LIST OF SPECIES FROM ZANZIBAR.

- Anoplocephala perfoliata* (Goeze 1782). Horse.
Dipylidium chyeri von Rátz 1897. Domestic cat.
Dipylidium oerley von Rátz 1900. Domestic cat.
Inermicapsifer zanzibarensis n.sp. Pouched rat (*Cricetomys gambianum* Eth).
Stilesia globipunctata (Rivolta 1874). Domestic goat.
Stilesia hepatica Wolffhügel 1903. Sheep.
Stilesia vittata (Railliet 1896). Camel (*Camelus dromedarius* L.).
Taenia hydatigena (Pallas 1766). Dog.
Taenia taeniaeformis (Batsch 1786). Cat.

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DESCRIPTION OF PLATE IX.

The following letters apply to all the figures: *c.s.*, cirrus-sac; *d.exc.*, dorsal excretory vessel; *d.v.*, dorso-ventral muscles; *e.*, egg; *e.c.*, egg capsule; *l.m.*, *l.'m.'*, *l.''m.''*, longitudinal muscles; *ov.*, ovary; *r.s.*, receptaculum seminis; *t.*, testes; *t.m.*, *t.'m.'*, *t.''m.''*, transverse muscles; *u.*, uterus; *u'*, remains of uterine wall; *v.exc.*, ventral excretory vessel; *y.g.*, yolk-gland.

Figs. 1-2. *Dipylidium oerley* von Rátz.

Fig. 1. Musculature of mature proglottis.

Fig. 2. Musculature of proglottis containing egg capsules.

Figs. 3-7. *Inermicapsifer zanzibarensis* n.sp.

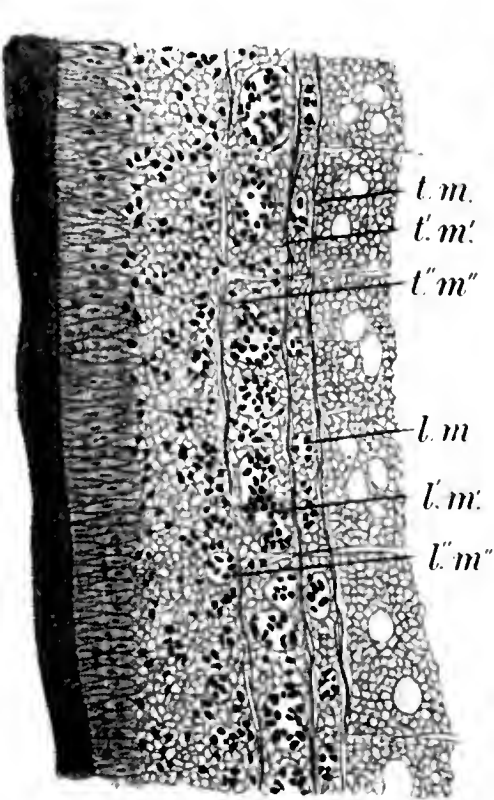
Fig. 3. Scolex.

Fig. 4. Mature proglottis.

Fig. 5. Formation of egg capsules, first stage.

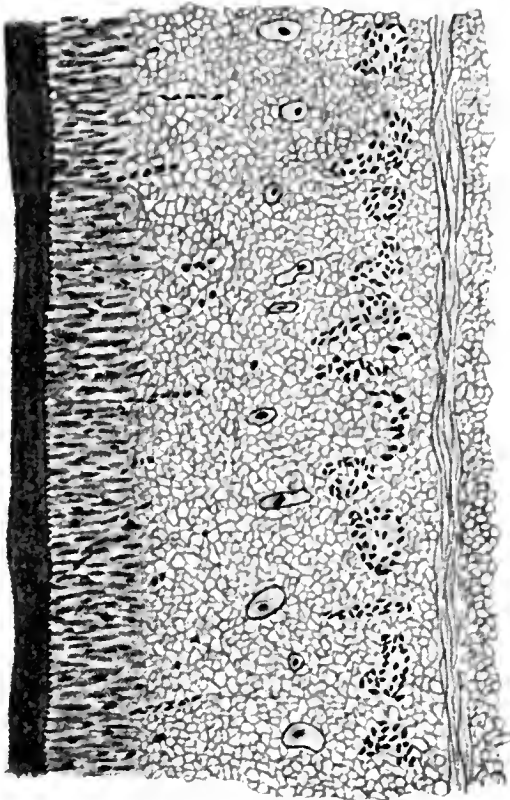
Fig. 6. Formation of egg capsules, second stage.

Fig. 7. Formation of egg capsules, third stage.



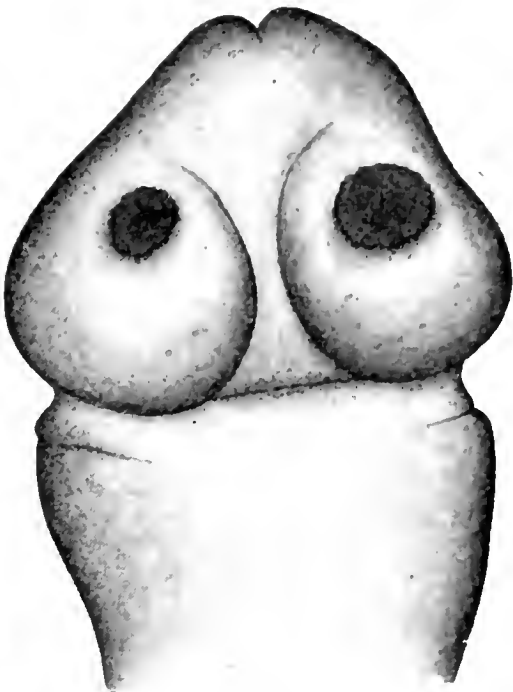
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Fig. 1



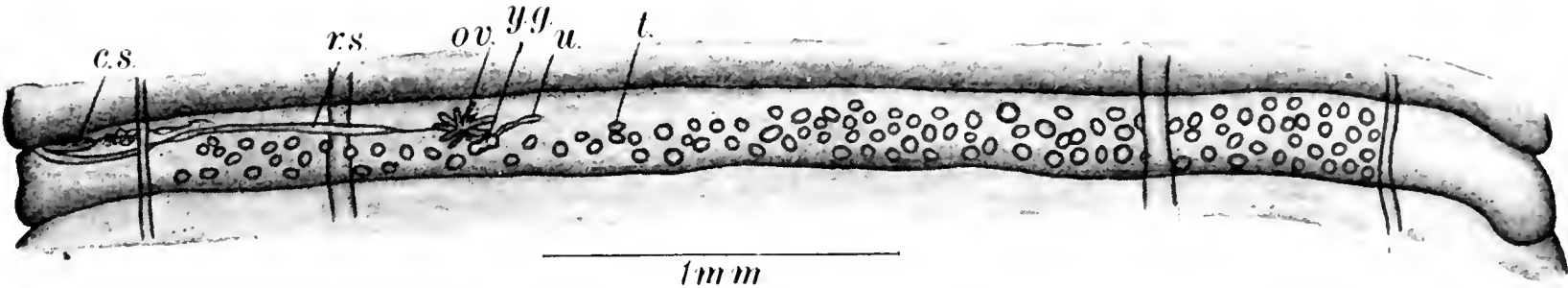
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Fig. 2



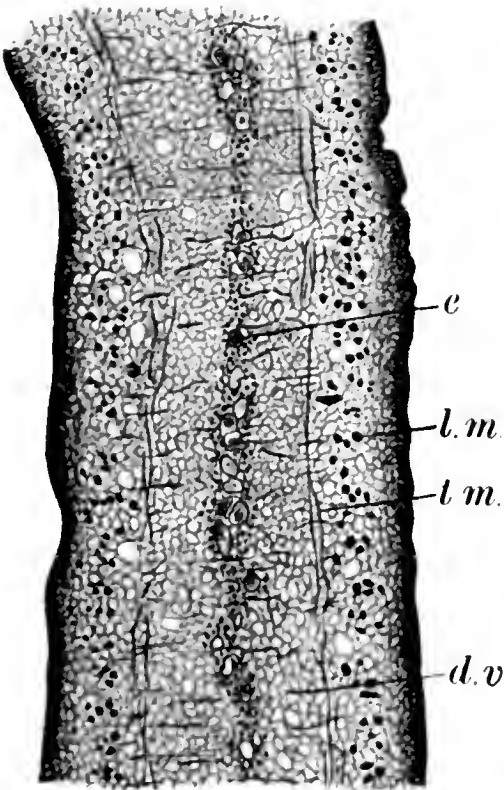
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Fig. 3



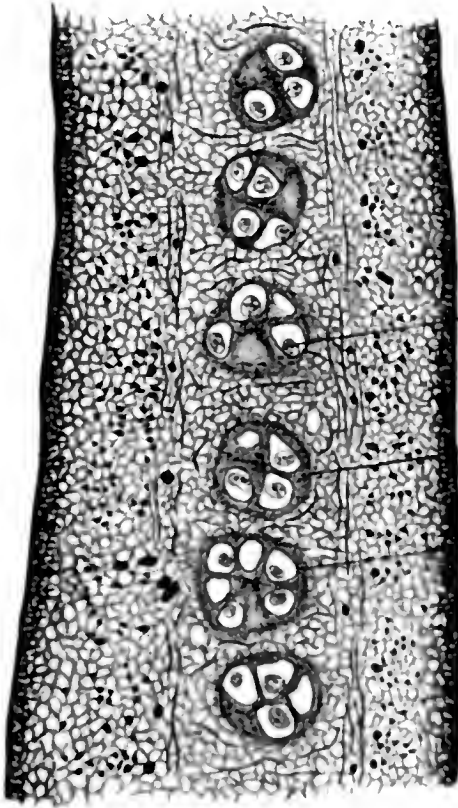
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Fig. 4



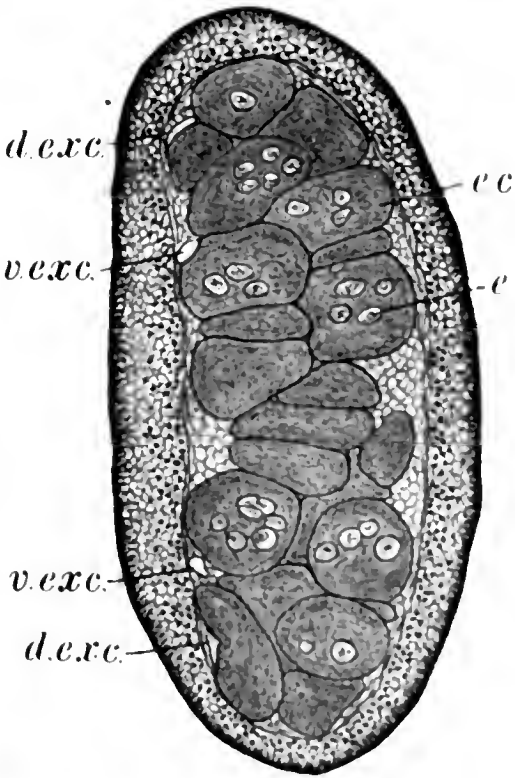
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Fig. 5



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Fig. 6



0.1mm

Fig. 7

THE EXCRETORY SYSTEM IN DIGENEA (TREMATODA).

IV. A STUDY OF THE STRUCTURE AND DEVELOPMENT OF THE EXCRETORY SYSTEM IN A CYSTOCERCOUS LARVA, *CERCARIA PEKINENSIS* NOV. SPEC.

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(With 6 Text-figures.)

THE first record of a cystocercous cercaria was published by Wright (1885). Since that time Braun (1891), Ward (1916), Faust (1918) and Pratt (1919) have contributed to the general morphology of the group. But apart from the collecting reservoir or bladder at the posterior end of the body and the lateral collecting tubules immediately emptying into the bladder the excretory system of these larvae has not been studied. It is the purpose of this paper to lay especial emphasis on this system.

DESCRIPTION OF *CERCARIA PEKINENSIS* NOV. SPEC.

This larva was obtained from the testis of *Vivipara lapillorum* (Heude), taken from the Grand Canal, outside the East Wall, and from the North Lake, Imperial City, Peking. At first it was not easy to distinguish this species from the large non-bilharzian furcocercous larvae, but a careful study soon made the differences apparent. The movement is characteristically that of the cystocercous larvae, namely, backward instead of forward. The furci are distinctly flapper-like. The posterior portion of the body is partially enveloped by the distal portion of the tail. This tendency of the tail to surround the body is characteristic of the group. In *Cercaria mirabilis*, *C. wrightii*, *C. anchoroides*, and *C. macrostoma* the body is completely enclosed in the anterior portion of the tail, while in *C. stephanocauda* the enveloping tissue is only slightly developed. In this as well as other particulars *Cercaria pekinensis* bears a striking resemblance to *C. brookoveri* (Faust 1918).

C. pekinensis has a body measurement of 0.7 to 0.74 mm. in length by 0.21 to 0.26 mm. in transverse diameter. The tail trunk is 1.5 mm long by 0.21 mm. in diameter while the furci are 0.57 mm. long by 0.16 mm. in transverse measurement. Neither body nor tail has any mammillations or other permanent differentiations of the integument such as characterise most of

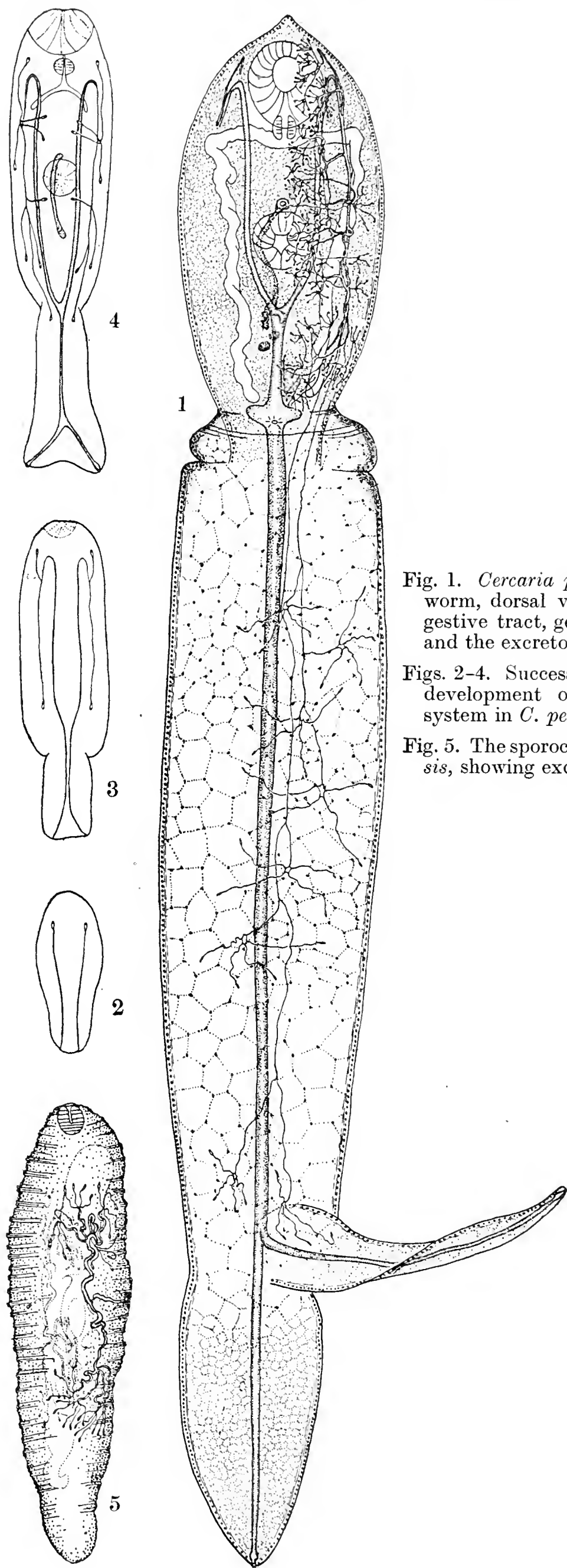


Fig. 1. *Cercaria pekinensis*, entire worm, dorsal view, showing digestive tract, genital cell masses and the excretory system.

Figs. 2-4. Successive stages in the development of the excretory system in *C. pekinensis*.

Fig. 5. The sporocyst of *C. pekinensis*, showing excretory system.

the described species of this group. The oral sucker measures 0.148 by 0.165 mm., while the acetabulum which is situated slightly anterior to the middle of the body measures 0.115 by 0.113 mm. The pharynx has a transverse diameter of 43μ . Anterior to the acetabulum is the conspicuous genital pore. From the pharynx the coeca first extend laterad and then posteriad, ending just anterior to the excretory bladder. These coeca are long narrow pouches as distinguished from the large thick-walled ones of the closely related species, *C. brookoveri*.

The genital organs are but poorly developed in *C. pekinensis*, even in the most mature individuals. However, the two testes have become differentiated and a lobate body anterior to them is connected by a heavy cord of genital-gland tissue to the genital pore anteriorly. In this respect *C. pekinensis* represents a type somewhat more mature than *C. brookoveri*, although it does not approach the degree of maturity of *C. macrostoma*.

Neither cephalic nor cystogenous glands have been seen in the larva. The body parenchyma consists of a close network of tissue. In the tail trunk, however, the parenchyma is extremely loose and vacuolated, with small condensation nuclei only at the interstices. On the other hand the furci have a close network of tissue like the body.

C. pekinensis develops in a sporocyst characterised by an anterior end with pharyngeal sphincter but without a gut. At regular intervals along the body the contracted sporocyst (Fig. 5) has annuli of integument and muscular tissue, which disappear, however, when the animal elongates. In this respect it is strikingly similar to the sporocyst of *C. brookoveri*. The sporocyst with its enclosed larvae has a rapid shuttle-cock movement.

There are two or more generations of sporocysts. Some sporosacs are found to contain nothing but developing sporocysts. Others contain both sporocysts and cercariae. Still others contain cercariae alone. Only a few flukes develop within the parthenita at one time. The tail becomes differentiated from the body soon after the germ-ball stage is passed; but the furci appear late. At the time when the furci first become distinguishable from the tail trunk the oral and ventral suckers are well developed. The pharynx is clearly marked off, but the digestive coeca, while readily observed, only extend a short distance posteriad. At this same stage there is a distinct genital cord extending longitudinally across the face of the acetabulum, suggesting an early sexual maturity in the worm which is not carried out in subsequent development.

No encystment of *C. pekinensis* has been observed nor does the worm readily part with its tail. It seems probable that the fluke passes directly into the subsequent host without a latent period.

THE EXCRETORY SYSTEM.

In studying *C. macrostoma* I noticed numerous flame cells connected with the ultimate capillaries of the excretory system but was unable to work out the pattern on account of the large portion of the body occupied by the mature genital complex. In *C. stephanocauda* I was able to count eight main groups of collecting tubules on each side of the body and in some of these groups I made but sixteen flame cells to each group. *Cercaria pekinensis* has proved very favourable for working out the entire excretory system, not only in the mature cercaria but in typical stages of development and in the sporocyst as well.

In the cercaria, the bladder at the posterior end of the body is compressed oval in shape with a dorsal pore. From the bladder a median trunk extends into the tail, dividing into two trunks somewhat anterior to the bifurcation of that organ. Each of these furcal branches runs to the end of the furcus where it opens to the exterior through a conspicuous pore. These caudal canals receive no lateral branches. They are drainage channels. Coming into the bladder from the anterior side is a single median canal which, when traced forward, divides about two-thirds the distance toward the acetabulum, the two branches of which run toward the oral sucker. They cross over the transverse portion of the corresponding coecum to the side of the oral sucker, in the mid-plane of which they turn back on themselves. As the secondary tubules they are recurrent the entire length of the body (see figures).

In its reversed course the secondary tubule receives eight main (tertiary) branches. The first, third, fifth, and seventh of these provide for excretory drainage of the dorsal portion of the worm, while the alternate ones care for the ventral side. Each of these tertiary tubules, when traced a short distance distad, is found to bifurcate, each fork of which has a definite field of function. This fork in turn receives four tubules at a common centre, and, finally, each of these receives four capillaries, for each of which there is a single solenocyte or flame-cell. Where each group of four tubules unites in a common centre to form the tubule of next higher rank there is a definite polygonal enlargement (see Fig. 6). This is, however, devoid of any concrement.

The anterior four main branches (tertiary tubules) are preacetabular, the fifth is acetabular, the sixth and seventh are postacetabular but within the body, while the eighth provides for drainage of the tail. This latter has an interesting distribution. One ramus cares for the anterior half of the tail trunk (Fig. 1) while the other not only drains the remainder of the tail trunk, but also the flapper of the corresponding side. This elaborate provision for ridding the tail of excretory wastes is apparently unique among cercariae. The furcocercariae have an analogous derivative from the posterior end of the body excretory system but there is no record of such an elaboration of the furcal branch in that group. The condition in the cystocercariae is probably explained by the extraordinary size of the tail common to all described species of the group.

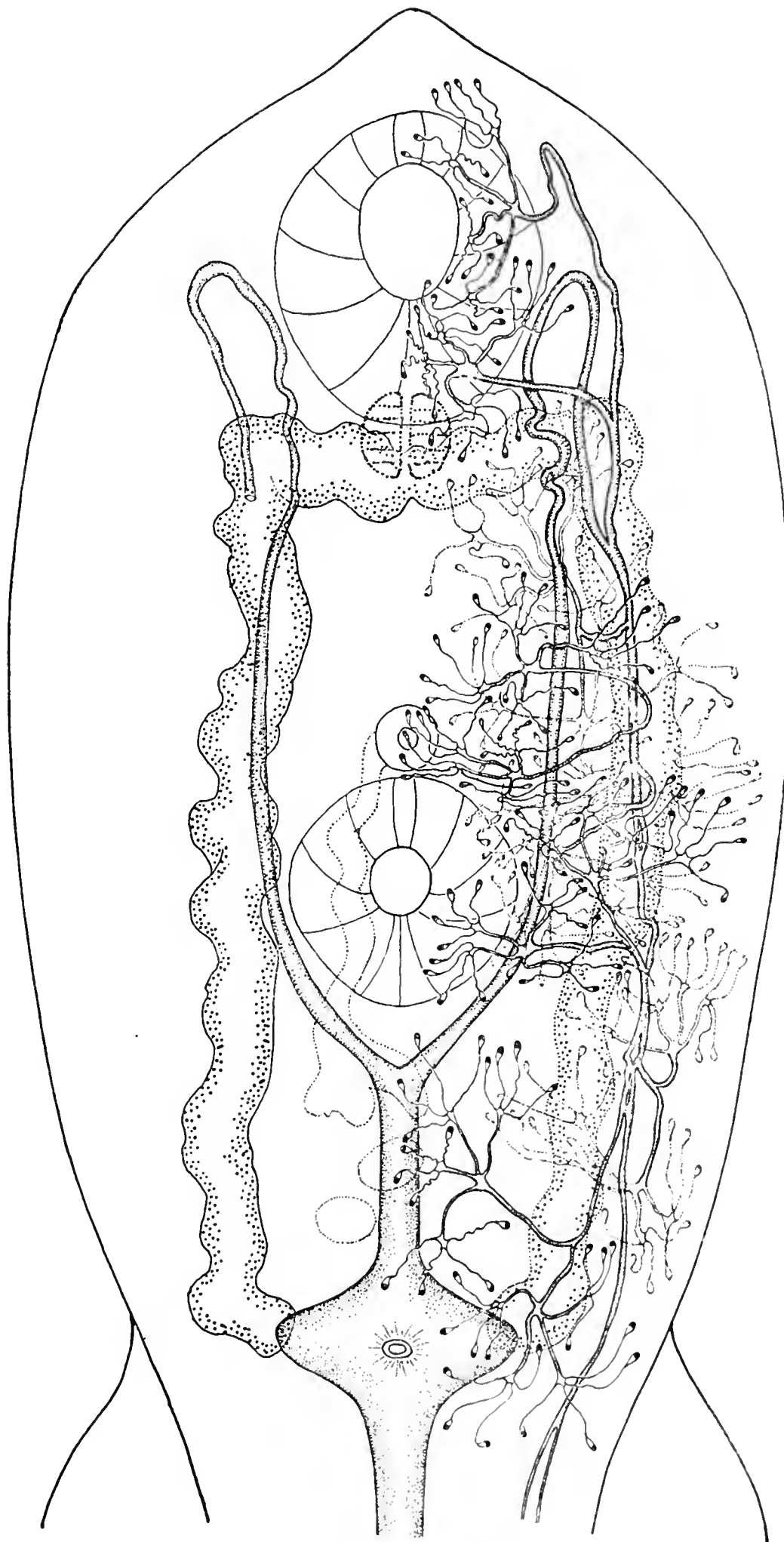


Fig. 6. Body of *Cercaria pekinensis*, enlarged to show pattern of excretory tubules.

Between the third and seventh branches (tertiary tubules) there are several islands of protoplasm (Fig. 6). A study of the younger stages indicates that these were originally lines of separation for the tertiary tubules.

Observation of the excretory system in consecutive periods of development in the fluke makes clear the process of successive differentiation. The young larva just beyond the germ-ball stage (Fig. 2) has a single drainage tubule for each side of the body, opening only at the posterior end and terminating anteriorly in a single flame-cell. As the larva develops and the tail region is definitely marked off, the caudal portion of each tubule (Fig. 3) approaches the median line. Meanwhile the flame-cell at the anterior end of the body has divided and the main tubule has become recurrent, so that at the end of this stage there is a single flame-cell in the region of the pharynx and a second flame-cell in a postacetabular position. The next stage (Fig. 4) shows a merging of the two canals in the tail trunk with a separation only in the furcal buds. Each of the two flame-cells in the body has given rise to four cells and the canals have split far backward. This double series of four flame-cells I regard as the fundamental pattern of the excretory system of *Cercaria pекinensis*. Later these tertiary branches separate from one another, providing the eight main branches of the system. By a subsequent bifurcation and two succeeding quadruple splittings the pattern of the developed cercaria is achieved.

In conformity with previous studies in this series (Faust 1919, 1919 *a*, 1919 *b*), the pattern of the system may be expressed as

$$[(2 \times 4 \times 4) + (2 \times 4 \times 4) + (2 \times 4 \times 4) + (2 \times 4 \times 4)] \\ + [(2 \times 4 \times 4) + (2 \times 4 \times 4) + (2 \times 4 \times 4) + (2 \times 4 \times 4)].$$

The primitive pattern may be designated by $\alpha + \beta$, while the fundamental pattern is

$$[\alpha^i + \alpha^{ii} + \alpha^{iii} + \alpha^{iv}] + [\beta^i + \beta^{ii} + \beta^{iii} + \beta^{iv}].$$

A study of the sporocyst shows that exactly the same pattern obtains. There are two pores, one on each side of the body with a tubule emptying through it. Each main tubule in turn receives an anterior and a posterior branch. Tracing the system further distad each secondary tubule gives place to four tertiary ones, while each tertiary one is the confluence of four capillaries with a flame-cell at each terminus (Fig. 5). It is seen that this pattern is precise, a replica, indeed, of the double fourfold division in each of the quaternary tubules of the cercaria. Taking the entire excretory system on each side of the sporocyst, one sees that it corresponds part by part with a single main (tertiary) tubule of the cercaria. Phylogenetically considered, the system in this species is, therefore, more conservative than is usual in *Digenea*. Since this same pattern probably obtains in *Cercaria stephanocauda*, it seems possible that the entire group of cystocercous cercariae may have the same fundamental common denominator,

$$[\alpha^i + \alpha^{ii} + \alpha^{iii} + \alpha^{iv}] + [\beta^i + \beta^{ii} + \beta^{iii} + \beta^{iv}].$$

It likewise seems significant that in the cercaria the pattern has been impressed

twice, for it is represented not only in the main tubules but also in each of the tertiary tubule afferents. Beyond this is the four-fold division into capillaries which apparently represents an addition to the fundamental formula. The more complete formula then is $(\alpha)^2 + (\beta)^2$, while $4(\alpha)^2 + 4(\beta)^2$ represents the flame-cell total for each side of the cercaria.

DISCUSSION.

The problem presented in this study is a fundamental one. The experimental biologist has shown that the organism obeys within limits certain definite laws, some of which, such as the cleavage of the embryo and the division of sex cells in the earlier stages, are mathematically precise and predetermined. The problem of the excretory system in the trematode is no less a problem in mathematical development. I have previously shown how this obtains in the amphistome group (Faust 1919), in the echinostomes, xiphidio-cercariae and furcocercariae of the distome group (Faust 1919 b). Cort has confirmed this in his several studies on distome species. Moreover, these larval studies are supported by the monumental work of Looss (1894, 1896) on adult forms. In unpublished studies of holostomes and aspidobothrids I have found the same definite plan of development.

It is of equal significance that the fundamental pattern of excretory tubules of a particular species (representing a group) obtains for the parthenita (sporocyst or redia) of that species. This has previously been demonstrated in *Cercariaeum mutabile* (Cort 1919), in the amphistome, *Cercaria convoluta* (Faust 1919) and in the monostome, *Cercaria spatula* (Faust 1919 b). The present paper shows it to obtain in *Cercaria pekinensis*. The pattern is apparently so potentially inherent in the protoplasm that, through the several generations, even profound alterations in other systems and tissues and, indeed, in habits of living, including temperature, tonicity, and H-ion concentration, have practically no effect on this system.

SUMMARY.

1. *Cercaria pekinensis*, a new cystocercous cercaria, is described.
2. The excretory system of the developed cercaria consists of a bladder, a drainage channel through the tail and a pair of anterior collecting tubules, each of which receives eight main (tertiary) branches. Each branch is composed of $2 \times 4 \times 4$ units. The posterior-most branch cares for the drainage of the tail, including the corresponding flapper.
3. A study of representative stages in development shows that the common denominator of the excretory system is $\alpha + \beta$, while the pattern of the developed cercaria is expressed by $4(\alpha)^2 + 4(\beta)^2$.
4. The fundamental pattern of the cercaria is likewise found in the sporocyst.
5. These data have a significant bearing on the application of mathematics to the problems of growth and development.

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A PROTOZOON PATHOGENIC TO MOSQUITO LARVAE.

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A BATCH of twenty-nine larvae of *Stegomyia scutellaris* Walker, were collected on 24 July 1920 from an old earthenware pot found in a Malay's garden. It was at once seen, even with the unaided eye, that many, some considerably paler than others, were characterised by a pearly-white opalescence of the tracheal gills at the hinder end of the body.

On microscopic examination it was found that the appearance was due to the presence of a large number of protozoa, which, though in most cases restricted to the gills, had in the paler larvae pervaded the body cavity, and the head even to the interior of the antennae.

The protozoa, which were under ordinary conditions pear-shaped and of considerable size, were usually to be seen careering most actively up and down within the gill as if seeking a point of exit from the larva, but in some cases were packed so tightly as to distend the gill. They were then immobile and spherical. One gill only was usually so distended, sometimes two, and occasionally three and four, but invariably when one gill showed the parasites in large numbers, the others showed them to a less extent.

It was almost impossible to form any correct estimate of the number of organisms present in any one gill. One hundred and fifty-seven were counted in a gill moderately packed with them, and in a gill fully distended it is probable that double that number might be present.

When in such cases a breach of surface of one of the gills was made with a fine needle a large number of the protozoa came out and raced about wildly under the field of the microscope, and it could then be determined that they were provided with flagella.

Evidence of their pathogenicity was afforded by the death in the course of a few days of the entire original brood of larvae. Some two or three, which had shown but few of the parasites, did indeed pupate, and parasites were seen moving slowly within, but none afforded imagoes.

Within a day or two after the death of a larva most of the parasites had left it, though a small number, spherical and probably encysted, remained aggregated together.

It is possible that the softening of the tissues consequent on the death of the larva may be necessary in some instances before the organisms, especially those in the tougher regions of the body, can make their escape. But escape does take place mainly from the larva, while yet alive, either by the rupture, or by the complete separation of the gills; for, on looking through a series of sickly larvae, it was noticed that often one, sometimes two and rarely all the gills were absent; and by placing larvae having the full complement of gills but showing infection, each in small separate bowls, the facts as to rupture and separation of the gills were verified.

Larvae did not seem to be greatly affected by the loss of one gill, and occasionally specimens which had lost all four were observed. None were ever known to pupate, though one larva without any gills managed almost completely to detach most of its skin in attempted pupation, then dying with the skin still enveloping its last two or three segments.

On the death of the last larva a fresh batch of fifty half-grown *Stegomyia* larvae were put into the water; most of these pupated and a few died, but showed no evidence of the presence of the protozoa. Thereafter until October 16, almost three months later, batches of the larvae were from time to time put in, keeping the number constantly between thirty and fifty, a little of the fluid in which they had occurred being added to make up for the evaporation of the original water.

During all these weeks there was a constant succession of pupations or deaths among the larvae and it was not until this date that the protozoa again showed evidences of their presence. It is perhaps noteworthy that during the last fortnight the bowl, which had been kept in a well-lighted situation, had been removed to a dark corner of the laboratory.

On October 16 three of the larvae, floating at the surface, were seen each with one milky-looking gill in which, on microscopic examination, the protozoa were found to be swarming, the other three gills being affected to a less degree.

An examination was then made of every larva and out of twenty-seven, thirteen were apparently healthy, while fourteen, some lacking one or more gills, showed the presence of the parasites to a greater or less extent.

The appearances presented by each of the affected larvae, most of which were approaching maturity, were as follows, the specimens being numbered 1-14:

(1) Alive; one gill showing extreme distension with organisms; one gill about half-full; other gills showing one organism each: very moderate body infestation, a few only here and there.—(2) and (3) As specimen (1).—(4) Dead: had lost one gill. In one of the remaining gills were about twenty of the parasites, spherical, immobile and apparently attached to each other, for they moved *en masse* when some of the living organisms, of which there were about ten, butted against them. In each of the other two gills were about an equal number of resting and actively moving organisms, about twenty in all. These teemed in the body.—(5) Alive; had lost two gills. The remaining gills showed

a moderate infestation with organisms all of which were active. There were a few in the body.—(6) Alive; all four gills were present, one half-full of parasites; one containing about twenty, the others about ten each; all very active: parasites present in great abundance in body especially posteriorly, all active.—(7) Alive; but moribund; lying flat on water: one gill only present. There were no organisms in it and there was no body infestation.—(8) Alive; had three gills only; no infestation either of these or of body.—(9) Alive; no gills at all: slight infestation of body: all organisms very active.—(10) Alive; only two gills were present; one full of granular material, one showing at base about seventeen organisms at rest; a few scattered throughout body.—(11) Alive; all gills were present and in each of three were two organisms, the fourth showing none: in the body were a few scattered organisms; not more than about ten in all.—(12) Alive; only one gill was present, containing about thirteen active organisms: none to be seen in body.—(13) Alive; half-grown, had two gills only; one full of organisms, one half-full; all active; showed a few, perhaps ten in all, in body.—(14) Alive; had one gill only; but marked infestation of the body, especially posteriorly.

ON A NEW CILIATE: *LAMBORNELLA STEGOMYIAE*
N.G., N.SP., PARASITIC IN THE BODY-CAVITY OF THE
LARVAE OF *STEGOMYIA SCUTELLARIS* WALKER
(DIPTERA, NEMATOCERA, CULICIDAE).

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(With 6 Text-figures.)

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I. INTRODUCTION.

THE material which forms the subject of this communication was derived from Dr W. A. Lamborn's collection, and was placed at my disposal through the kindness of Dr Guy A. K. Marshall, Director of the Imperial Bureau of Entomology, London. It comprised eight larvae of *Stegomyia scutellaris* Walker, which had been preserved in formaldehyde solution (10 per cent.), and the specimens were mounted individually on microscope slides in the same medium.

Examined in transmitted light, the preparations showed the body-cavities of the larvae to contain large numbers of the parasites, a feature which had already been described by Lamborn (1921).

From the nature of the preparations, it may be imagined that it was a matter of no small difficulty to carry out the detailed examination of the cellular structure of the organisms. It was, of course, necessary to remove specimens from the body of the host for the purpose of cytological study. The procedure which I adopted was as follows: the larvae were first examined *in toto*, and sketches were made in order to show the general distribution of the parasites in the larval body.

Some of the larvae were then divided into three pieces, and transferred from the formalin medium in which they were mounted into distilled water, in which they were allowed to remain for about an hour. They were then stained in a strong solution of haemalum, differentiated in alcohol (30 per cent.) to which a little hydrochloric acid had been added, and washed for a few minutes in tap-water. The pieces were then passed slowly through the graded alcohols, cleared in clove oil followed by xylol, and then into very liquid Canada balsam. Some of the pieces were mounted whole, but others were teased up in a drop of balsam, using a high magnification of the binocular microscope and very fine needles, with the object of liberating the stained parasites from the body of the host. When the parasites were well scattered in the balsam, a cover-glass, which had been momentarily immersed in xylol, was dropped on.

Other portions of the larvae were embedded in paraffin and sections cut, but the latter proved to be less instructive than the teased preparations.

Of the eight larvae examined, two proved to be free from parasites; they had been included in the lot on account of their mutilated condition, both having been found dead, with three gills missing, a condition which it was thought by Lamborn might be attributed to a parasitic infection. Five of the larvae showed the presence of the Ciliate parasite, but the sixth was the host of a fungal parasite with which I am dealing in another paper.

II. *LAMBORNELLA STEGOMYIAE*, n.gen., n.sp.

The ciliate about to be described is a new form, and I have named it in honour of its discoverer—Dr W. A. Lamborn¹.

The parasite is found distributed throughout the body-cavity of the host, from the head to the anal segment, and even penetrates to the respiratory siphon and the gills. Fig. 1 shows the appearance of the posterior extremity of a parasitised larva as seen by transmitted light. Sections of the same larva show no obvious damage to the internal organs, with the exception of the fat-body which, in places, has disappeared completely, while the parts which remain are reduced to a few cells devoid of fat-droplets.

The condition of each of the five parasitised larvae was as follows:

Larva No. 1. One of the four gills missing; body filled with parasites, as represented in Fig. 1.

Larva No. 2. Parasites scattered throughout the body-cavity; all four gills present, one of which is tightly packed with parasites, another moderately invaded, and the other two with very few ciliates; parasites very numerous in the respiratory siphon, surrounding the tracheal trunks.

Larva No. 3. Ciliates numerous in the posterior segments, scattered in the head and the remaining segments; two of the gills are missing, one of the remaining gills contains numerous parasites in its proximal portion only, the

¹ The name *Lambornia* is preoccupied for an Insect (Lepidoptera, Geometridae: Prout, *Genera Insectorum*, 1912, p. 235).

other is free from infection. As will be seen later, this larva yielded *Lambornella* cysts.

Larva No. 4. Contains few parasites; labelled by Lamborn "early infestation with protozoal parasites."

Larva No. 5. Found dead, with only one gill remaining; few parasites scattered throughout the body-cavity.

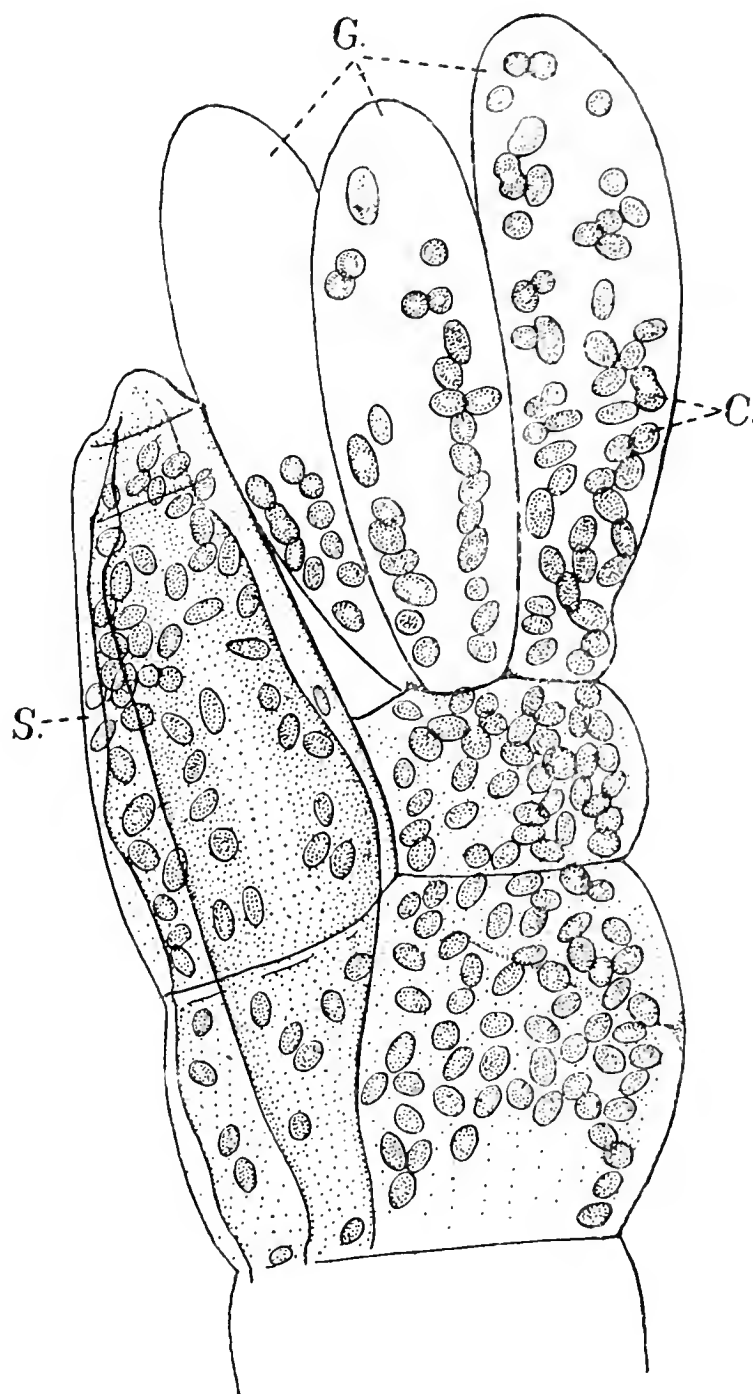


Fig. 1. Larva of *Stegomyia scutellaris* Walker, posterior end of the body, siphon and gills showing by transparency a very great number of parasitic ciliates (*Lambornella stegomyiae*). The contours of the larva are represented schematically, all the accessory organs and sensory hairs being omitted.

In the living state, the body of *Lambornella stegomyiae* is described by Lamborn (1921, p. 213) as being pear-shaped; in a few cases specimens fixed in formalin still retain this shape, but, more often they are elongately oval, ranging in length from 50μ to 70μ , with a maximum diameter of 20μ to 30μ . The body surface is uniformly covered with cilia, densely disposed, and arranged in longitudinal parallel rows. The ectoplasmic layer is not clearly defined; the endoplasm is very granular. The macronucleus (Figs. 2 and 3, *M.*) is spherical, from 10μ to 13μ in diameter, stains deeply and uniformly in

haemalum or haematoxylin, showing neither granules nor vacuoles. The micronucleus (Figs. 2 and 3, *m.*) appears as a small chromatic granule, 2μ to 3μ in diameter, and usually lies in a small peripheral depression of the macronucleus. Its apparent position depends on the orientation of the body of the parasite, and varies from a marginal to a central position, but careful examination shows that its position is superficial on the macronucleus, sunk into a shallow depression or notch. In a few cases in which I have observed the

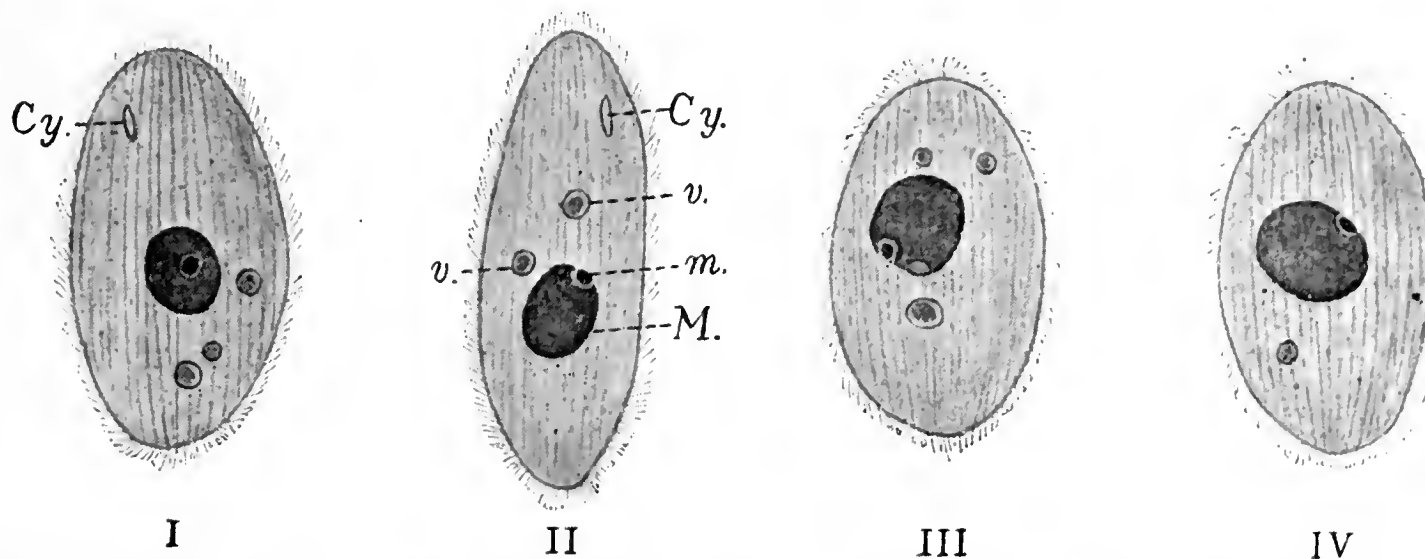


Fig. 2. *Lambornella stegomyiae*, showing the cytostome, *Cy.*; micronucleus, *m.*; macronucleus, *M.*

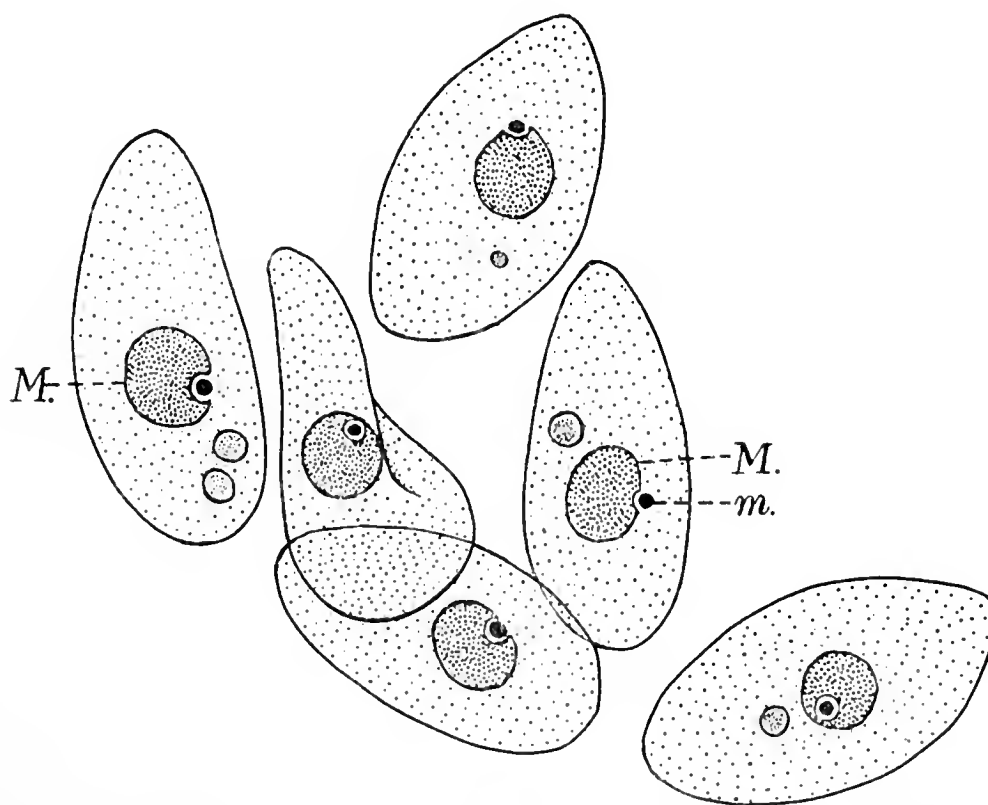


Fig. 3. *Lambornella stegomyiae*. A group of six specimens of this ciliate as seen in one field of the microscope. Letters as in Fig. 2.

micronucleus widely separated from the macronucleus, the latter invariably showed an empty depression, from which the micronucleus had doubtless been displaced accidentally. In addition to the two nuclei, the protoplasm contains one to four vacuoles which stain uniformly with basic dyes. Notwithstanding its purely parasitic mode of life, *Lambornella* possesses a small cytostome. The cytostome is difficult to see in fixed and stained specimens, and I thought at first that I was dealing with a ciliate belonging to the group of the Astoma,

which are all known to be parasites of animals. Finally, however, by careful search under a high magnification, I have succeeded in demonstrating its presence. It appears as a small, lozenge-shaped, refractile spot (Fig. 2, I and II, *Cy.*), interrupting the longitudinal striations of the body surface. It is ventral in position and forms a small groove, situated at a distance of 10μ from the anterior end of the body, and measuring 5 to 6μ in length and 2 to 3μ in width. The cytostome is most easily seen when viewed in profile.

III. REPRODUCTION.

Reproduction of *Lambornella stegomyiae* takes place by simple, transverse fission. The micronucleus first divides mitotically, and when the two daughter micronuclei have appeared, the macronucleus undergoes an amitotic division, accompanied by a constriction of the body and fission of the protoplasm (Fig. 4, *A* and *B*). I have not seen any evidence of conjugation.

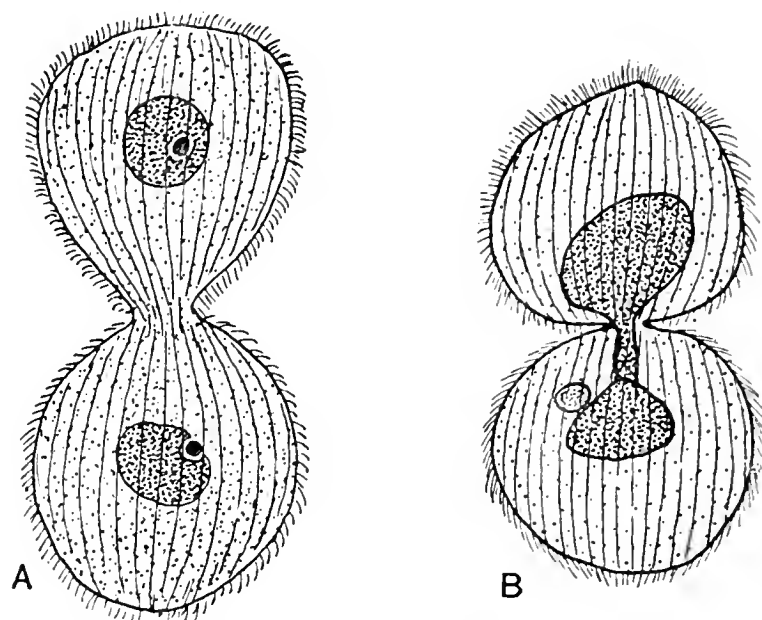


Fig. 4. *Lambornella stegomyiae*. Two dividing specimens of the ciliate.

IV. ENCYSTED STAGE.

Among the hundreds of parasites seen within the bodies of the *Stegomyia* larvae, no cysts were present; all the ciliates were in the active or trophic phase. One of the larvae only (see p. 218, No. 4) showed the cystic form of *Lambornella*. The external surface of the cuticle of this larva was studded with small, transparent, hemispherical vesicles, each of which enclosed a contracted mass of protoplasm (Fig. 5). These I first thought to be epizootic organisms such as are commonly found upon the bodies of fresh-water Arthropods. In stained preparations, however, the protoplasmic mass was seen to contain a nucleus so closely resembling in its structure that of *Lambornella* as described above, that no doubt remains in my mind: these vesicles are unquestionably the cysts of the ciliate.

The cysts measure from 30μ to 40μ in diameter and 20μ in height (Fig. 6). The protoplasmic mass (22μ to 32μ in diameter) contains a contracted macronucleus (8μ to 10μ in diameter) with a peripheral depression in which the

small micronucleus is borne. In a few individuals, I was still able to perceive the faint superficial striation of the protoplasm and one or two basophile vacuoles. Several cysts showed a clear vacuole ranging from 3μ to 8μ in diameter. The wall of the cyst is very thin and does not show any definite structure.

From the foregoing, it is evident that the parasite passes through a period of rapid multiplication within the body of the *Stegomyia* larva, after which

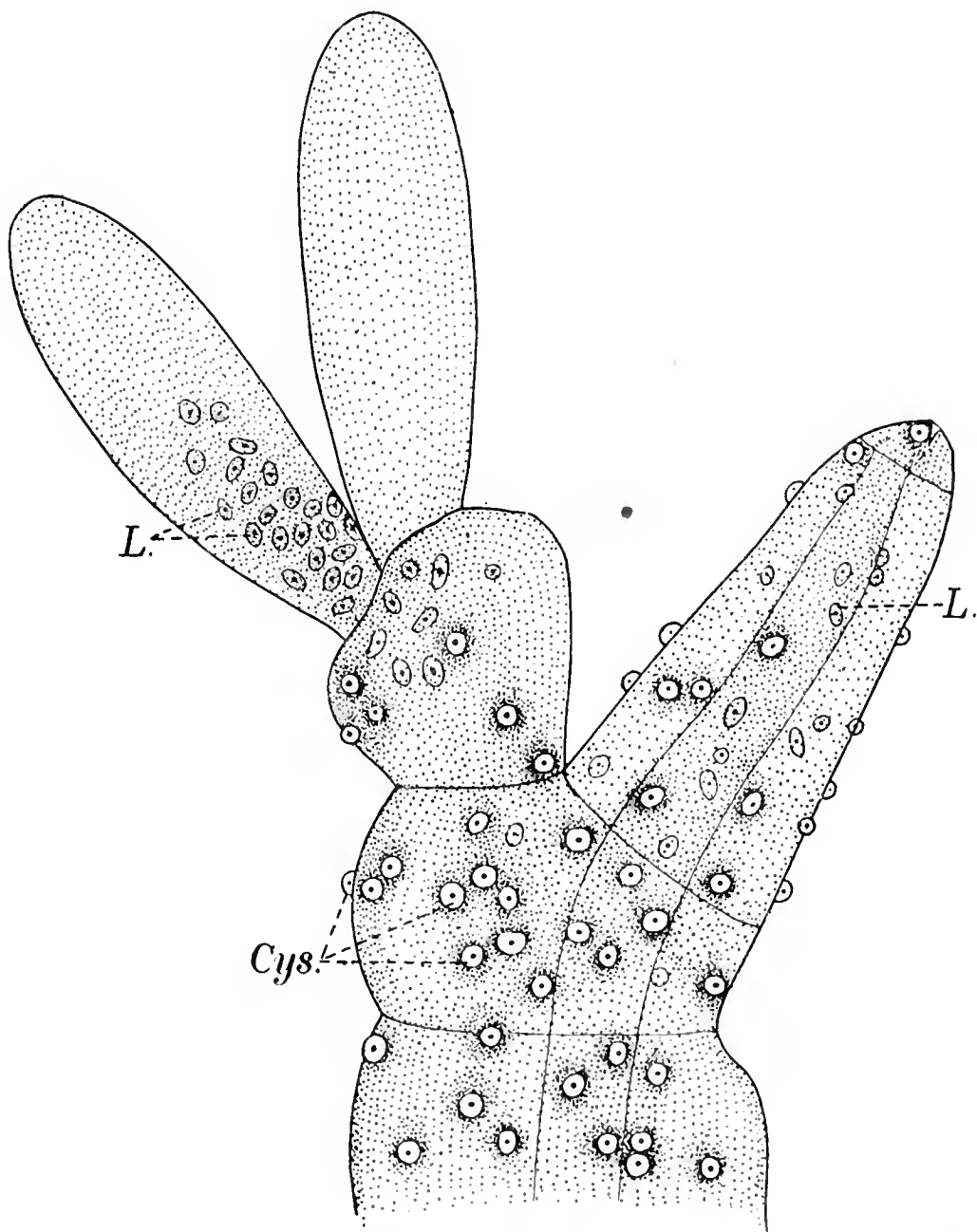


Fig. 5. Larva of *Stegomyia scutellaris* Walker, posterior end of the body showing by transparency several specimens of active stages of *Lambornella stegomyiae*, *L.* and many cysts of this ciliate, *Cys.*, fixed upon the cuticle of the larva.

it leaves its host and fixes itself on the external surface of the cuticle (and, possibly, to any adjacent solid object), and there becomes encysted. The parasites probably leave the body of the host through artificial ruptures of the body-wall, and as the gills are the most fragile of the appendages of the larva, it appears to be likely that the parasites reach the exterior through the openings which are left after the gills have broken away (see Lamborn, p. 214). In connection with this, it is interesting to note that larva No. 4, which was covered with cysts of *Lambornella*, had lost two of its gills.

The formation of external cysts attached to the body of the host has been observed in other ciliates parasitic in the body-cavity of Arthropods. According to Balbiani (1885), *Collinia circulans* (Balbiani) Cépède 1910, which lives as a parasite in the body-cavity of *Asellus aquaticus*, after passing through a phase of active multiplication in the body-cavity of the host, escapes through ruptures in the antennae and attaches itself to the external cuticle of the host, or to the surrounding filaments of the alga *Cladophora*, where it becomes encysted. A similar mode of encystment has been observed by Cépède and

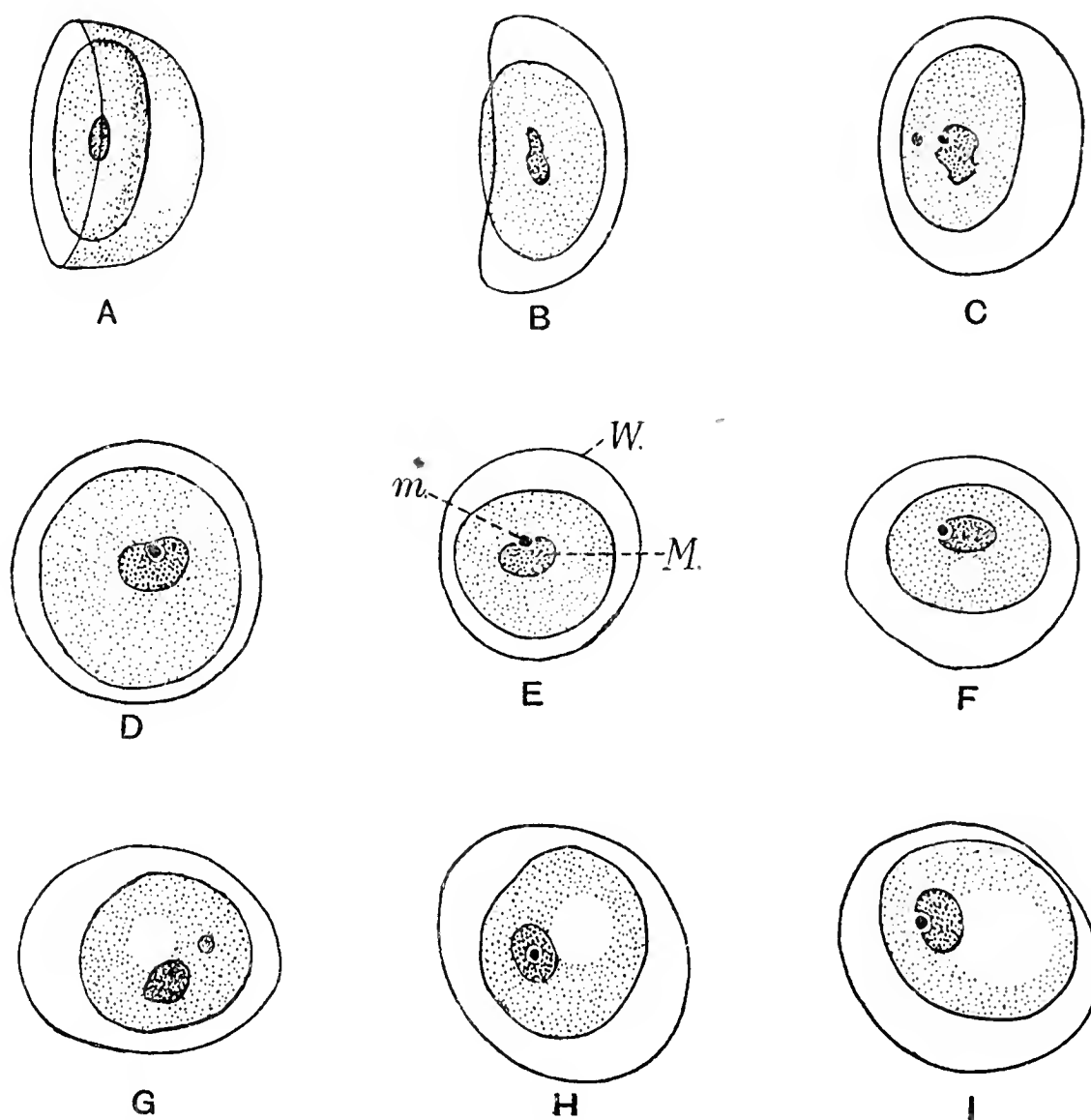


Fig. 6. *Lambornella stegomyiae*. Different forms of the cysts of this ciliate; *m.* micronucleus; *M.* macronucleus; *W.* wall of the cyst.

Giard (1910) in *Perezella pelagica* Cépède, a ciliate parasite found in the body-cavity of marine Copepods.

V. MODE OF INFECTION.

In his note (p. 214), Lamborn records that he has succeeded in infecting healthy *Stegomyia* larvae by keeping them with infected living and dead specimens of the larva. Infection is probably acquired through the mouth in the case of *Lambornella*, but it should be mentioned that Balbiani failed to infect healthy *Asellus* by feeding them upon the cysts of *Collinia*. It is possible that the failure was due to the fact that the cysts were unripe.

It is highly probable that parasitic ciliates such as *Collinia* and *Lambornella* undergo a process of multiplication within the cyst-wall, comparable to that which is observed to occur in *Ichthyophthirius*, a parasite of fresh-water fishes, and that only those cysts filled with young ciliates, which are capable of penetrating the walls of the alimentary canal of the host, are infective when ingested by the host.

That the cysts of *Lambornella* must pass through some developmental phase before they become infective, is supported by Lamborn's experience, in that he obtained successful results in his infection experiments not less than three months after the death of the larvae which he used as a source of infection.

VI. DIAGNOSIS OF *LAMBORNELLA*, n.gen.

Diagnosis: a holotrichous parasitic ciliate. *Body* elongate oval, length 50μ to 70μ , maximum width 20μ to 30μ . *Ciliation* dense. *Cytostome* small lozenge-shaped. *Macronucleus* spherical, 10μ to 12μ in diameter; *micronucleus* 2μ to 3μ in diameter, lying in a peripheral depression of the macronucleus. *Protoplasm* granular, with few basophil vacuoles. Multiplication takes place by simple transverse fission into two equal parts. *Cysts* hemispherical, 30μ to 40μ in diameter, 20μ high, transparent and structureless, attached to the host's cuticle.

One species known:

Lambornella stegomyiae, n.sp.

Diagnosis: as that of the genus.

Habitat: the body-cavity and gills of *Stegomyia scutellaris* Walker (Diptera, Nematocera, Culicidae).

Description based on material collected by Dr W. A. Lamborn at Kuala Lumpur, Federated Malay States.

VII. APPENDIX.

(a) *The Ciliate Parasites of Insects.*

The only example of a ciliate, parasitic in the alimentary canal of insects, which has been recorded hitherto, is *Nyctotherus*, the hosts of which are adults of *Blatta*, *Gryllotalpa*, and *Hydrophilus*, and the larva of *Oryctes* (see Bütschli, 1899, p. 1721).

To the best of my knowledge, *Lambornella stegomyiae* is the first and only instance of a ciliate parasitic in the body-cavity of an Insect.

(b) *The Ciliate Parasites of Arthropods in general.*

It is a matter of some surprise to find how few ciliate parasites of Arthropods are known: I append the following list:

1. *Anophrys maggi* Cattaneo, parasitic in the blood of *Carcinus maenas*.
The species is very insufficiently described.

2. *Collinia branchiarum* (Stein, 1852) Cépède 1910, parasitic in the gills of *Gammarus pulex*.
3. *Collinia neoniphargi* Cépède 1910, parasitic in the circulatory system of *Neoniphargus moniezi*.
4. *Collinia circulans* (Balbiani, 1885) Cépède 1910, parasitic in the body-cavity of *Asellus aquaticus*.
5. *Perezella pelagica* Cépède 1910, parasitic in the body-cavity of Copepoda (Calanidae), *Clausia elongata* Boeck., *Acartia clausi* Giesbrecht, and *Paracalanus parvus* Claus.
6. *Uronema rabaudi* Cépède 1910, parasitic in the body-cavity of *Clausia elongata* and *Acartia clausi*, also in the dead bodies of these forms.
7. *Nyctotherus* sp.? in the hind-gut of *Blatta*, *Gryllotalpa*, and *Hydrophilus* adults, and the larva of *Oryctes*; also in *Iulus* (Myriapoda) (see Bütschli, 1899, p. 1721).
8. *Lambornella stegomyiae*, n.gen., n.sp., parasitic in the body-cavity of the larva of *Stegomyia scutellaris* Walker.

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ON A NEW TYPE OF FUNGUS: *COELOMOMYCES*
STEGOMYIAE N.G., N.SP., PARASITIC IN THE BODY-
 CAVITY OF THE LARVA OF *STEGOMYIA SCUTELLARIS*
 WALKER (DIPTERA, NEMATOCERA, CULICIDAE).

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(With 7 Text-figures.)

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I. LOCALISATION OF THE PARASITE IN THE BODY
 OF *STEGOMYIA* LARVA.

AMONG six parasitised larvae of *Stegomyia scutellaris* Walker, collected by Dr W. A. Lamborn in the Federated Malay States, five contained a ciliate which I have described (*Parasitology*, XIII. p. 216) under the name of *Lambornella stegomyiae* n.g., n.sp., while the remaining larva harboured a new parasitic fungus which forms the subject of this communication. The larva had been fixed and was preserved in formaldehyde solution (10 per cent.) and was labelled: "Larva of *Stegomyia scutellaris* Walker. Infestation with protozoal parasite and a luxuriant growth of *Vorticella*. Kajang," by Dr Lamborn. When the larva was examined under a low power the surface of its body was found to be extensively covered with tufts of *Vorticella*, whilst its interior harboured an enormous number of parasites which in the gills and the posterior segments were packed in solid masses completely filling these parts (Fig. 1). As seen by transparency the parasites are oval in shape, 37·5 to 57 μ long and 20 to 30 μ in diameter, surrounded by a more or less thick yellowish wall. In their external appearance, size, and position in the host, they are so surprisingly similar to *Lambornella* that it was at first quite natural to take them for the resting stages or cysts of this ciliate. It is sufficient to compare Fig. 1 of this

paper with Fig. 1 (p. 218) of the paper on *Lambornella* to appreciate this misleading resemblance. The detailed study of the structure of this new parasite, however, showed that we were dealing not with the cysts of *Lambornella*, but with a totally different organism belonging to the Fungi.

For the study of this new parasite which I propose to name *Coelomomyces stegomyiae* n.g., n.sp., the best results were obtained (a) by teasing up pieces of the larva in lactophenol, either pure or after the addition of a drop of cotton-blue, and (b) by studying sections of the larva stained in glychaemalum or iron haematoxylin and eosin.

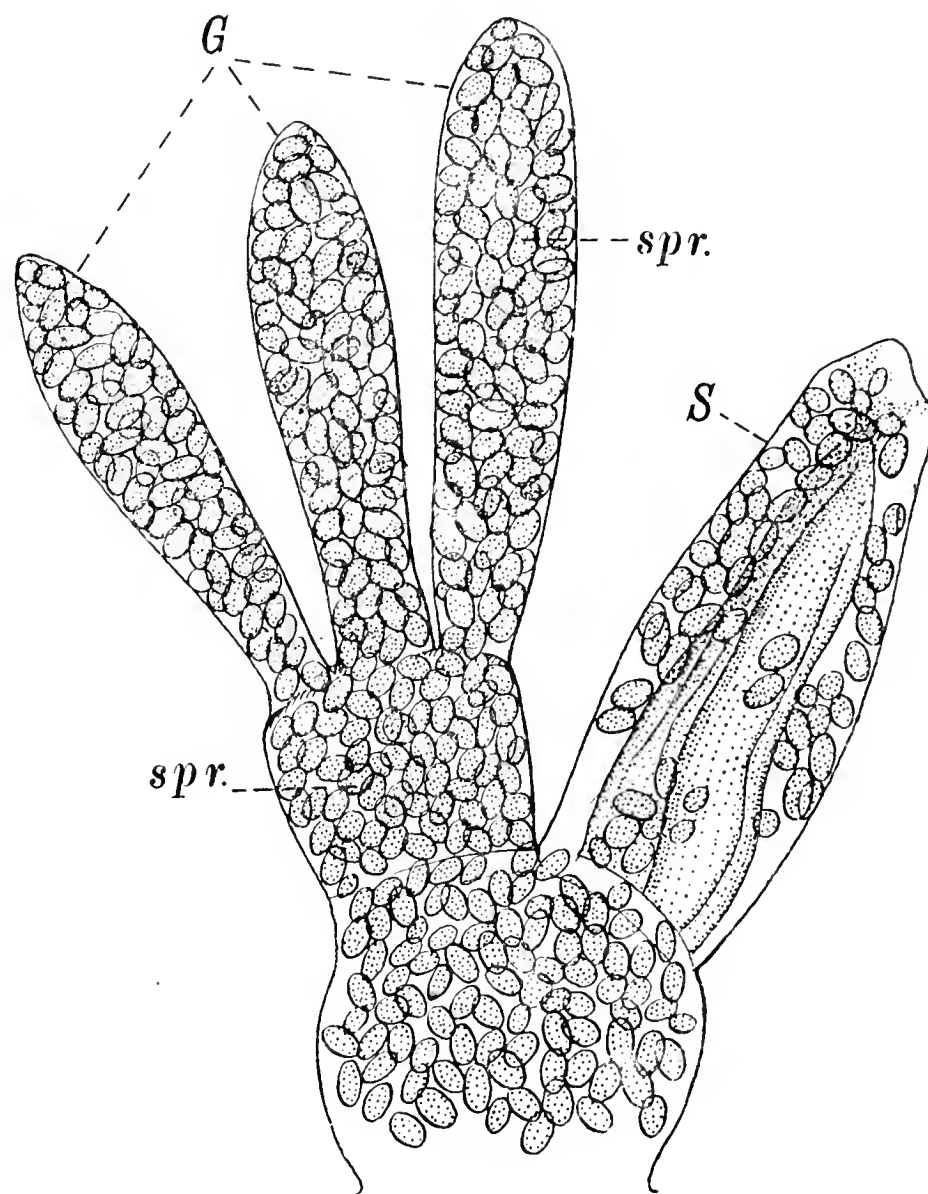


Fig. 1. *Stegomyia scutellaris* Walker. Posterior extremity of larva with three gills, *G*; and the respiratory siphon, *S*; packed with sporangia—*spr.* of *Coelomomyces stegomyiae*.

The sections show that, although the larva is very heavily infected and lacking one gill, its internal organs are apparently healthy, but for the fat body which has completely disappeared (Fig. 2).

II. MYCELIUM OF THE PARASITE.

Portions of the parasitised larva, examined in the manner described, showed not only the oval bodies previously referred to, but also fragments of true mycelium. There is little mycelium in the body-cavity, only a few branches being seen in sections, but the mycelium is well-developed immediately around the viscera, especially the midgut (Fig. 4) and the five anterior intestinal coeca,

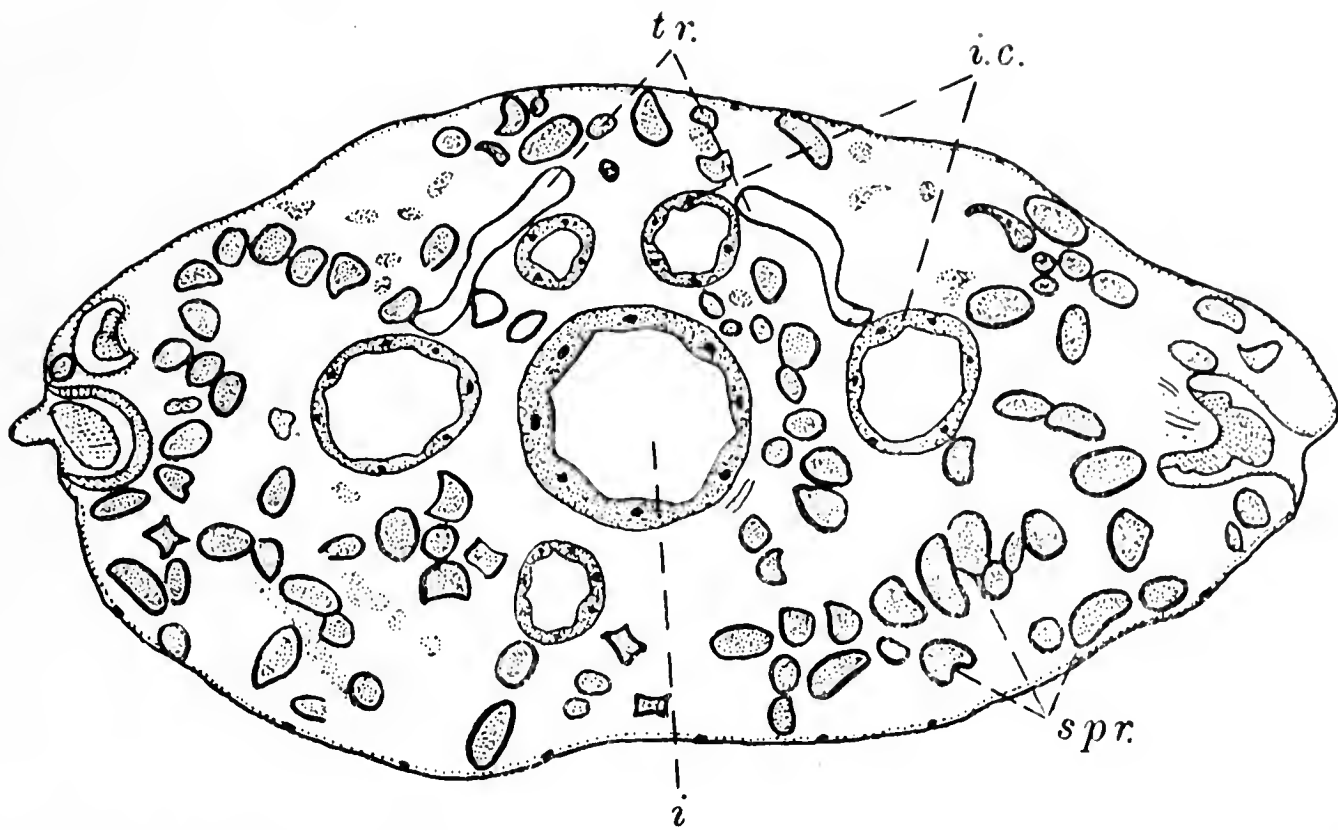


Fig. 2. *Stegomyia scutellaris* Walker. Transverse section of an abdominal segment of the larva showing the sporangia, *spr.*, of *Coelomomyces*; *i*, midgut; *i.c.*, intestinal coeca; *tr.*, tracheal trunks.

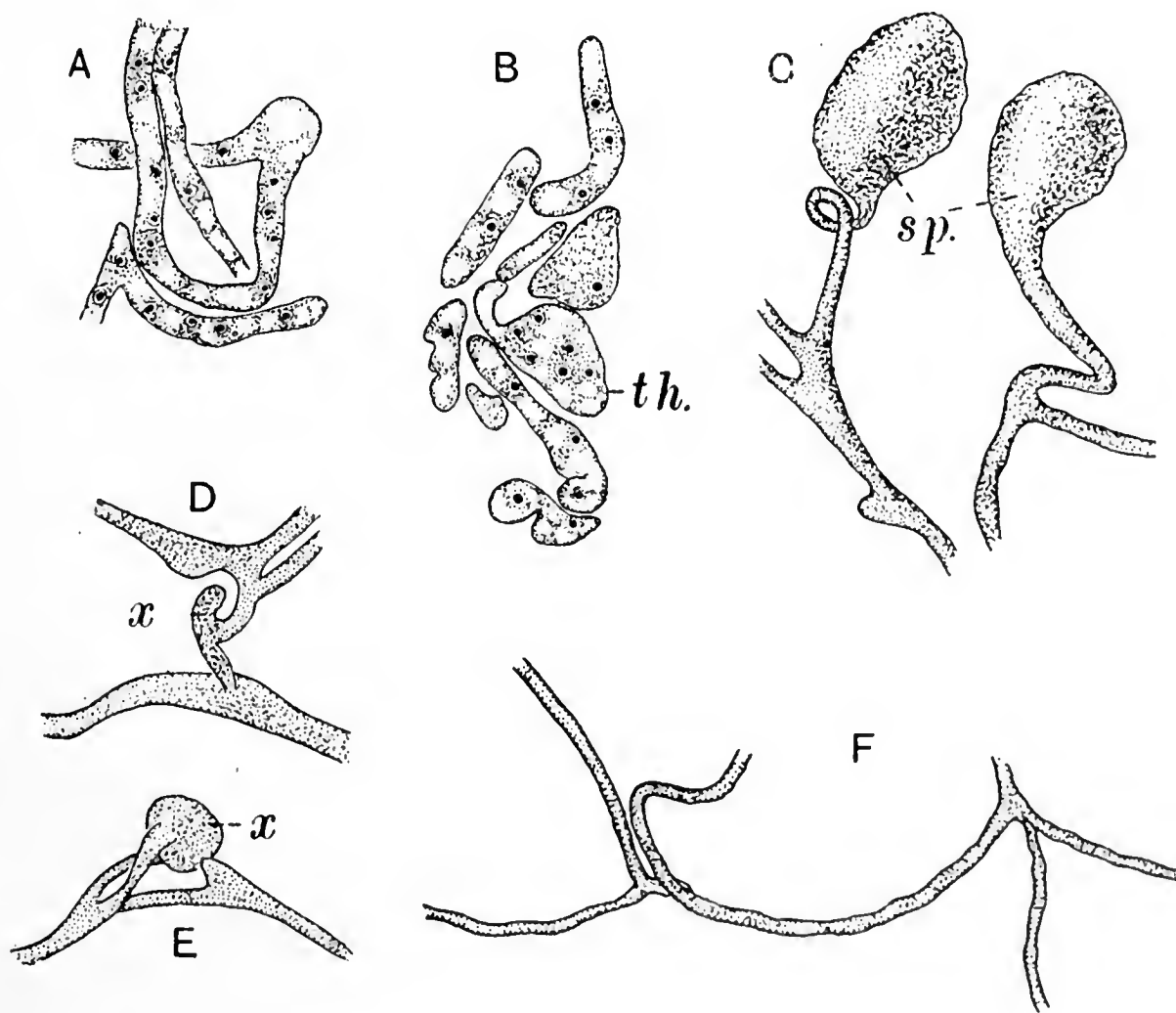


Fig. 3. *Coelomomyces stegomyiae*. A and B, sections of the mycelium stained in glychaemalum: *th.*, terminal thickenings which become transformed into sporangia; C, fragments of mycelium with terminal swellings in a more advanced stage; D and E, fragments of mycelium showing transverse branches with diverticula or spherical thickenings, *x*; F, the ordinary form of mycelium.

forming two to three concentric layers, so closely attached to the organs, that in some places it is difficult to separate the tissues of the host from the surrounding mycelium. The latter is also well-developed beneath the hypoderm of the host, where it is covered with the pigmented remains of the peripheral cells of the fat body (Fig. 5 *A*). Examined in lactophenol, the fragments of mycelium show numerous ramifications, the branches varying from 2 to 6 μ in thickness; while some fragments are more regularly ramified and about 3 μ thick (Fig. 3 *F*), the others are composed of entangled branches with irregular thickenings which seem to be formed by a confluence of two or more branches; in such places the mycelium attains a width of 5 to 6 μ (Fig. 4 *my*).

The main branches of the mycelium are often connected by short transverse branches which, in places, show a small diverticulum or a spherical thickening (Fig. 3 *D* and *E*, *x*). The mycelium, examined *in toto* in lactophenol-cotton blue, or in stained sections, did not show any transverse walls and is therefore unicellular. Its nuclei are very chromatic, crowded in places, and scattered elsewhere. Each nucleus is surrounded by a dense layer of protoplasm, which in the other parts of the mycelium is filled with vacuoles of various sizes and shapes (Fig. 3 *A* and *B*).

III. DEVELOPMENT AND STRUCTURE OF SPORANGIA.

The majority of the branches show terminal thickenings of various sizes; in some branches they are hardly noticeable, while in the others they form a pronounced terminal swelling 30 to 35 μ long and 20 to 22 μ thick (Fig. 3 *C*, *sp.*). The contours of these terminal dilatations are somewhat irregular and their surface often shows a series of very fine transverse wrinkles. These thickened portions are very difficult to stain *in toto*, but in sections they show the same structure as that of the ordinary mycelium, with the difference that they contain a larger number of nuclei, and the protoplasm is more vacuolated (Fig. 5 *A*). At a later stage of development the terminal swellings become separated from the mycelium and are found free within the insect's body-cavity, their length varying from 32 to 65 μ . At first they possess a structure identical with that of the terminal swelling of the mycelium, but they appear to increase in size after becoming free in the host's perivisceral fluid. The number of their nuclei varies with their size.

At a later stage these bodies become more regularly oval; the peripheral layer of protoplasm grows denser (Fig. 5 *C*) and the external wall thickens (Fig. 5 *D*, *w.*). The nuclei now increase in number, undergoing several divisions, and the protoplasm becomes more dense and basophile. The wall thickens progressively and assumes a complicated structure, ending in the development of sporangia, the form of the parasite that is most frequently found in the perivisceral cavity of the host. The sporangia constitute the main mass of parasites which fills the posterior segments of the host (see Fig. 1). When removed from the larva the sporangia appear as oval bodies measuring 37.5 to 57 μ in length and 20 to 30 μ in width, flattened on one side and convex on

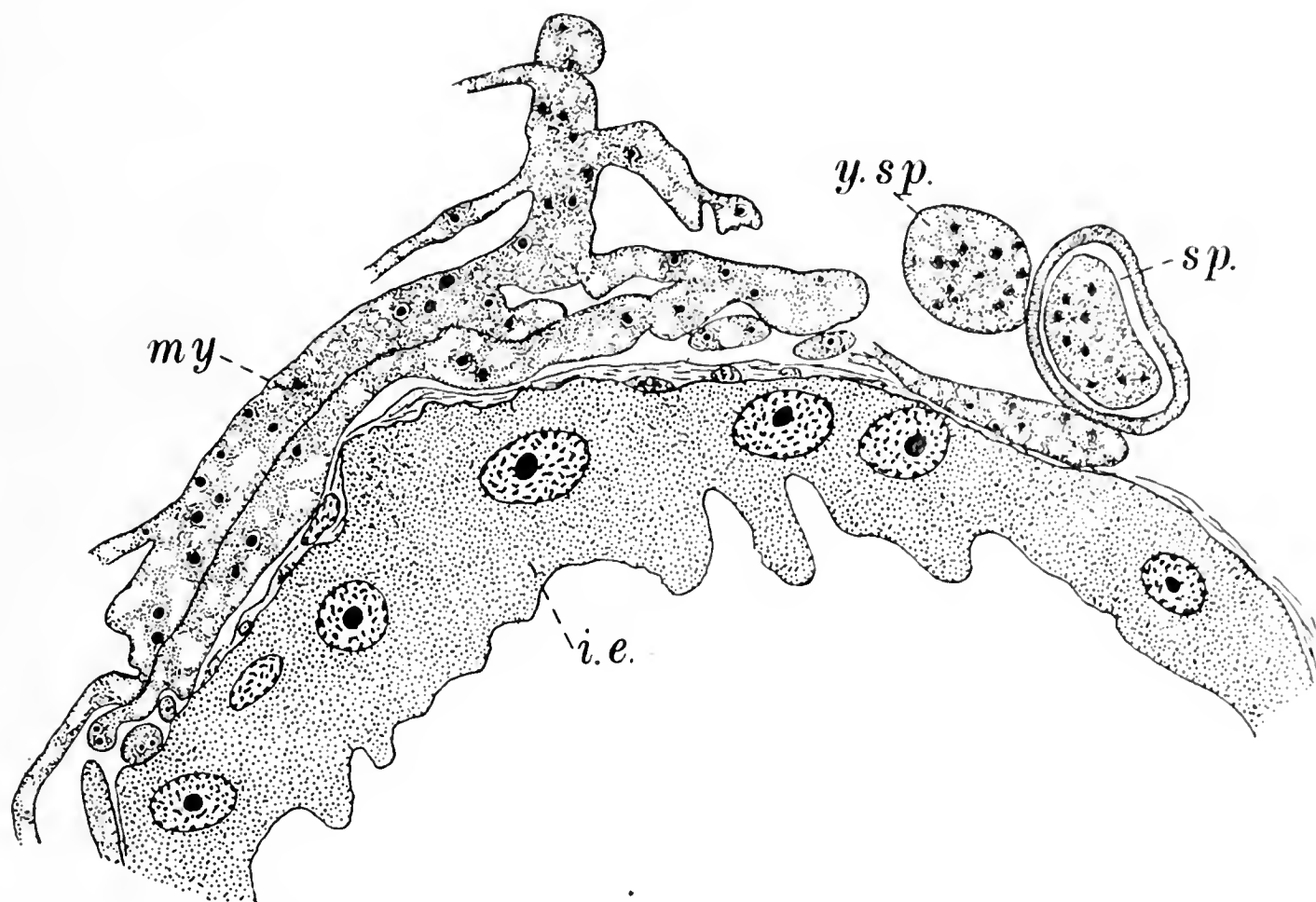


Fig. 4. *Stegomyia scutellaris* Walker larva; transverse section of the midgut showing the intestinal epithelium, *i.e.*, surrounded by a mycelium, *my*, of *Coelomomyces*; *y.sp.*, young sporangium; *sp.*, sporangium with thickened wall.

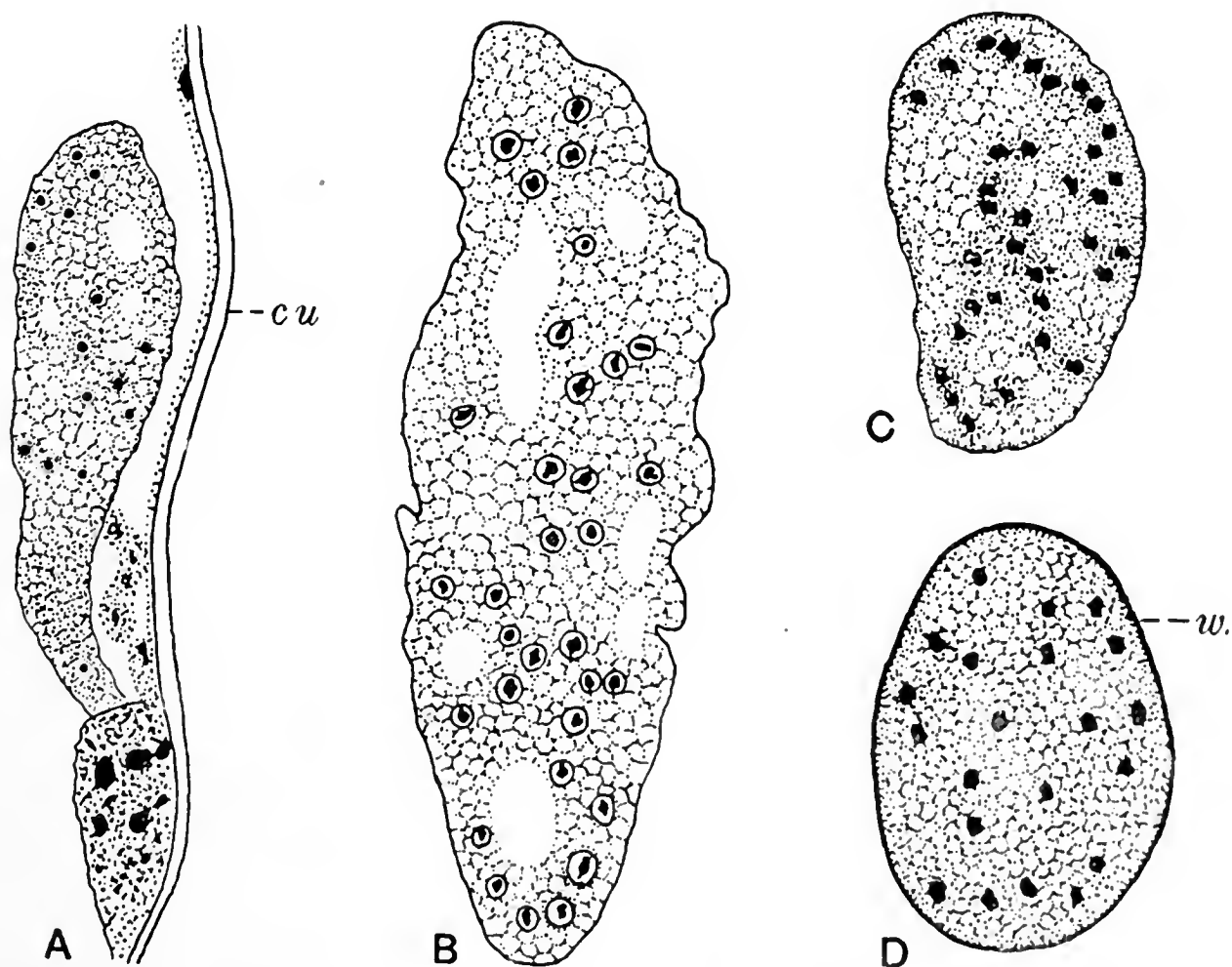


Fig. 5. *Coelomomyces stegomyiae*. *A*, section of a terminal thickening of mycelium which develops into a sporangium; *cu.*, cuticle of the larva; *B*, section of a terminal thickened portion of mycelium at a more advanced stage when it is detached from the mycelium and is free in the perivisceral fluid of the host; *C*, section of an early stage in development of a sporangium showing the condensation of the peripheral protoplasm; *D*, young sporangium showing the beginning of the thickening of the wall, *w.*

the other (Fig. 6 *A*). The wall of the sporangium when highly magnified shows a very fine granular structure and many small clear lenticular spots (Fig. 6 *B*, *a.*); the convex side shows a very fine line running from pole to pole.

Sections of the sporangium show that the wall consists of two distinct layers: (1) an internal, thin, structureless layer, $0.7\ \mu$ thick (Fig. 7 *A*, *en.*), and (2) an external layer (Fig. 7 *A*, *ex.*) 1.7 to $2\ \mu$ thick, of a yellowish colour, and showing numerous apertures (*a.*) already referred to as clear spots on the surface of the sporangium. By pressing a sporangium in a drop of water between the slide and a coverglass a number of small oily drops are seen to escape from the apertures of the external wall.

IV. DEVELOPMENT AND STRUCTURE OF SPORES.

The young sporangium is filled with very dense and basophile protoplasm, containing a great number of small nuclei (Fig. 7 *B*). As the sporangium

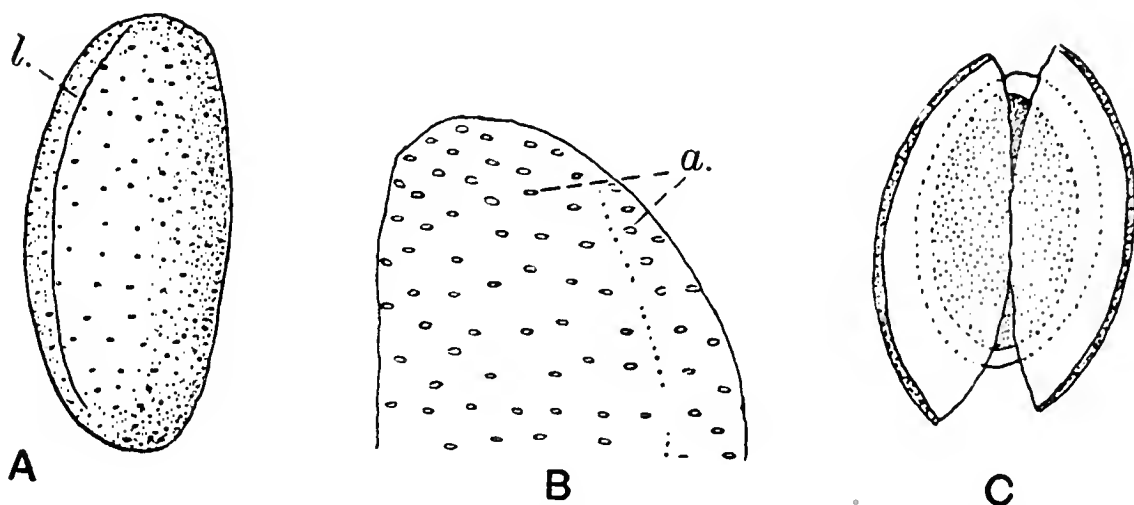


Fig. 6. *Coelomomyces stegomyiae*. *A*, a completely formed sporangium showing the clear line, *l.*, upon its convex side; *B*, sporangium showing the distribution of small apertures, *a.*, upon its external wall; *C*, breaking up of a sporangium under pressure.

develops the protoplasm divides into many portions, each of which surrounds a nucleus (Fig. 7 *C*) and gives rise to as many small spherical cells as there were nuclei (Fig. 7 *D*). These cells, $3\ \mu$ in diameter, become elongated, with a more or less central nucleus (Fig. 7 *E* and *F*). The sporangia filled with these lozenge-shaped spores, $5\ \mu$ long and $1\ \mu$ in diameter, were not very numerous, and they represent the last stage of the parasite which I could find in the host. Unfortunately the available material was insufficient for a more detailed study of these spores. Their shape, however, and the fact that they probably represent the final infective stage of a parasite of an aquatic host suggest that these spores are provided with one or two flagella and are the actual flagellisporos or zoospores which are so characteristic of some groups of lower fungi; this supposition could be readily demonstrated by an examination of living material.

V. PROBABLE MODE OF INFECTION.

The escape of these spores probably results from the rupture of the sporangium along the clearly marked line of cleavage that runs along its convex surface. I was able to observe such rupture of the sporangium by compressing

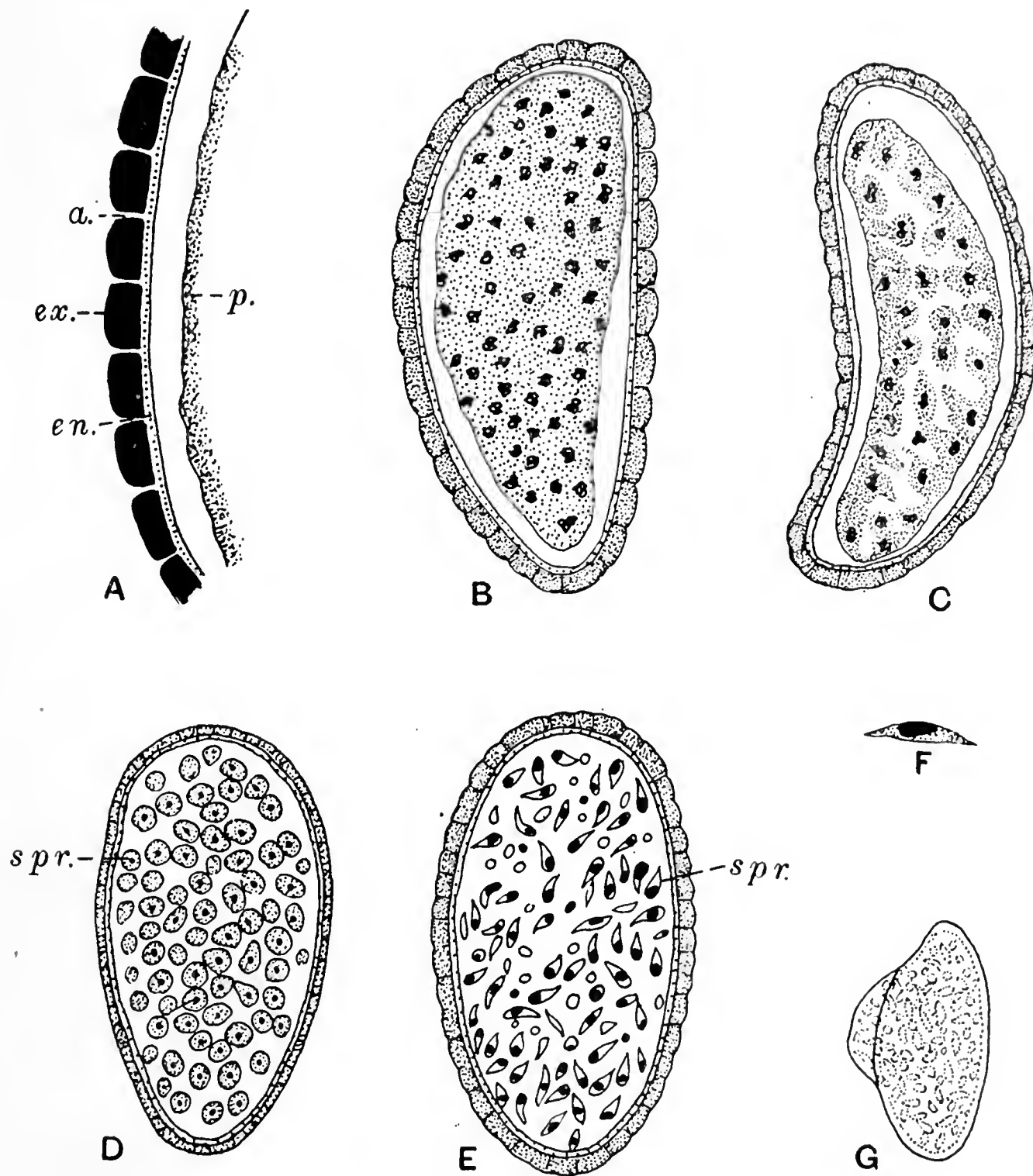


Fig. 7. *Coelomomyces stegomyiae*. *A*, section of the wall of sporangium: *a.*, apertures; *en.*, internal structureless membrane; *ex.*, external thick perforated membrane; *p.*, central protoplasmic content; *B*, section of an unripe sporangium; *C*, section of sporangium showing the beginning of the breaking up of its content into separate cells; *D*, sporangium with a thin wall and content divided into a great number of spherical cells, *spr.*; *E*, section of a sporangium showing the completely formed spores, *spr.*; *F*, spore (or probably a zoospore) from a sporangium similar to that represented in Fig. *E*; *G*, a small sporangium with spores, seen *in toto* the external layer having ruptured and the internal thin membrane bulging out from the slit.

it between the slide and a coverglass. When the external wall of the sporangium is ruptured, the thin internal wall bulges out and by its rupture allows the spores to escape. It is possible that the protruded internal wall of the

sporangium is capable of perforating the chitin of its host, whereby, as in some parasitic Olpidiaceae, the spores are conducted out of the host into the water.

It should be mentioned that two kinds of sporangia were found by me in the body-cavity of the *Stegomyia* larva: (1) sporangia with a very thick wall (Fig. 7 *A, B, C* and *E*), and (2) sporangia with a thin wall (Fig. 7 *D* and *G*), and this independent of the stage and the content of sporangia. It is possible that the latter serve for immediate reproduction, while the former represent a resistant or resting form of the parasite.

VI. SYSTEMATIC POSITION.

It is difficult to allocate this organism to its systematic position. Since the mycelium of *Coelomomyces* is devoid of transverse walls (*i.e.* it is unicellular), it would appear to belong to the Phycomycetes and it undoubtedly shows some resemblance to the Chytridineae¹. In this group, *Physoderma* (Cladochytriaceae) and *Catenaria* (Hyphochytriaceae) have a more or less similar development of the sporangia within the tissue or body-cavity of the host and they possess a more developed mycelium than other Chytridineae. The structure of the sporangia of *Coelomomyces* differs, however, in many respects from that of the Chytridineae and its mycelium appears to be better developed than is the case in any known representative of this group of Fungi. The systematic position of *Coelomomyces* cannot be finally established until more abundant and living material is available for a detailed study of its structure. It is still necessary to determine (1) the structural character of the mycelium during the early stages of infection, before it is transformed more or less completely into sporangia; (2) whether conjugation occurs, and at what stage of development of the parasite; (3) the structure of the spores; (4) the mode of their liberation from the body of the host and, finally, (5) the mode of infection of a new host and the formation of the first mycelium.

Several other matters of general interest still await solution, and, first of all, it is desirable to know if the parasite is confined to but one species of *Stegomyia* or if it infects other species of Culicidae or perhaps other Arthropods.

Acknowledgement. My thanks are due to Mr F. T. Brooks, M.A., University Lecturer in Botany, Cambridge, for his helpful suggestions in connection with this study.

VII. PARASITES OF MOSQUITO LARVAE RECORDED BY VARIOUS AUTHORS.

Fungi: 1. W. G. Liston (1901) mentions an ectoparasitic fungus resembling *Trichophyton* (?) attacking *Anopheles* larvae in Ellichpur.

2. According to Vaney and Conte (quoted by Dyé, p. 15) *Culex pipiens* larvae are sometimes destroyed by a fungus *Botrytis bassiana* (which causes destruction of silkworms).

¹ Schröter, J. (1897). Chytridineae, Ancylistineae, Saprolegniineae in Engler and Prantl: *Die Natürlichen Pflanzenfamilien*.

3. Léger and Duboscq (1902) found in *Anopheles* larvae at Campo di Loro, Corsica, a filamentous fungus similar to that previously noticed by Perroncito.

4. Laveran (1902) describes a parasitic yeast discovered by him in the coelomic cavity of *Anopheles maculipennis* from Rio-Tinto (Spain). According to Dr Macdonald this yeast is probably transmitted from the larva to the adult.

5. A fungus *Coelomomyces stegomyiae* Keilin parasitic in the larva of *Stegomyia scutellaris* is described in the present paper; the parasitised host was found by Dr Lamborn at Kajang, Federated Malay States.

Bacteria: A bacterial parasite resembling *Leptotrix buccalis* was discovered by Perroncito (1899, quoted by Howard, Dyar and Knab) as pathogenic to *Anopheles maculipennis* in Turin, Italy. It infests the larva, passes into the pupa, and destroys the imago soon after it emerges.

Spirochaeta: *Spirochaeta culicis* Jaffé is described by Jaffé (1907) as infesting the alimentary canal of undetermined mosquito larvae collected near Berlin. A similar organism was found by Sargent and Sargent (1906) in *Anopheles maculipennis* larvae in Algeria.

Sporozoa: *Gregarina*. *Lankesteria culicis* (Ross 1898) Wenyon 1911 was found by R. Ross in mosquito larvae at Secunderabad and by Marchoux, Salimbeni and Simond (1903) in *Aedes calopus* at Rio de Janeiro. The complete life history of this gregarine (from *St. fasciata*, Bagdad) we owe to Wenyon. Léger and Duboscq (1902) found a *Diplocystis* in mosquito larvae in Corsica.

Schizogregarina: A schizogregarine belonging to the genus *Caulleryella* Keilin (1914) has been recently described by Hesse (1918) under the name *Caulleryella anophelis* n.sp. parasitic in the alimentary canal of *Anopheles bifurcatus* larvae in Dauphiné, France.

Microsporidia: 1. *Nosema stegomyiae* was found by Marchoux, Salimbeni and Simond (1903) as pathogenic to *Stegomyia fasciata* larvae.

2. *Thelohania legeri* Hesse was described by Hesse (1904) as parasitic in *Anopheles maculipennis* larvae.

Thel. magna Kudo and *Thel. illinoisensis* Kudo were found by Kudo (1921) in the larvae of *Culex pipiens* and *Anopheles punctipennis* respectively.

Flagellata: Léger (1902) describes *Crithidia fasciculata* n.sp. parasitic in the gut of *Anopheles maculipennis*; the parasite is transmitted hereditarily.

Ciliata: A new ciliate, *Lambornella stegomyiae* Keilin was discovered by Lamborn in the body-cavity of *Stegomyia scutellaris* larvae, from Federated Malay States.

Nematoda: An *Agamomermis* was described by Stiles (1899, quoted by Howard, Dyar and Knab) from *Culex nemoralis* near Leipzig.

Dr Smith (quoted by Howard, Dyar and Knab, 1912, p. 163) records that in 1902 Dr H. P. Johns found the young of an intestinal worm in an *Anopheles* larva.

Gendre (1909) found larval nematodes at Labé, French Guinea, infesting *Stegomyia fasciata* larvae. It inhabits the body-cavity and leaves the host when the latter is ready to pupate; the larva then dies.

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STUDIES ON THE INTESTINAL PROTOZOA FOUND
IN THE TERMITES OF JAPAN.

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(With Plates X to XV and 5 Text-figures.)

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INTRODUCTION.

THE protozoa found in the intestines of termites constitute one of the most interesting but less illuminated fields in protozoology. The amount of work done by a number of authors in several different parts of the world is, however, already fairly large, especially valuable papers having appeared in recent years, so that some light has been thrown on the field. Nevertheless,

important questions regarding morphology, physiology, systematics, etc., still remain either insufficiently studied or almost untouched. Having myself been occupied for some years past with these interesting organisms, harboured by three species of termites in Japan proper and Formosa, and having made observations which seem to be of some interest, I propose in this paper to describe the chief results of my studies.

It was at the Government Institute for Infectious Diseases (Director: Professor S. Kitasato) in Tokyo that my studies were begun. After nearly a year, my post was changed to the Institute of Science, Government of Formosa, where my studies were continued for nearly two years. An account of my work, written in Japanese, has already been published in the Government Report of the Committee for the study of damage done by termites (1916 and 1917). I intended to publish a detailed report in English, but have been prevented from doing so until now. The publication of this paper has been rendered possible, owing to the unusual kindness of Mr Clifford Dobell, F.R.S., in improving my manuscript and preparing it for the press. He also made it possible for me to consult all the literature and gave me much valuable advice. This, together with my further studies made after the appearance of the first report, makes this paper differ in several respects from the original. Had it not been for Mr Dobell's hearty help, the present paper would have remained as an imperfect manuscript for some time longer. I therefore express my warmest thanks to Mr Dobell for his kindness. I have also to acknowledge my indebtedness to the staffs of the Institute for Infectious Diseases of Tokyo and the Institute of Science of Formosa, for their assistance during the course of my work, and to the Medical Research Council for affording me laboratory accommodation and aid which enabled me to complete my studies in London.

PART I. DESCRIPTIVE.

1. HISTORICAL REVIEW.

The first author who mentioned the peculiar intestinal protozoa of termites was apparently Lespès (1856), who found "Infusoria" in *Leucotermes lucifugus*. In two papers, which appeared in 1877 and 1881, Leidy gave the first detailed descriptions of three forms which he had discovered in *Termes flavipes* of North America, referring them to three genera, viz. *Trichonympha*, *Pyrsonympha*, and *Dinenympha*. A very short note by Seip (1881), recording that he had found organisms like those described in Leidy's paper (1877) in termites of New Jersey, appeared shortly afterwards. In 1885 appeared Saville Kent's short paper dealing with species found in a Tasmanian termite, and also with the forms studied by Leidy. Subsequently Grassi and his co-workers made valuable contributions to the advancement of our knowledge of the group. After publishing several short notes in 1885, 1888, and 1892, in a famous work on the termites published in 1893 conjointly with Sandias,

Grassi gave revised descriptions of the organisms studied by him up to that date. The host insects which he had examined were *Calotermes flavicollis* and *Termes lucifugus*, and the organisms were referred to seven genera, viz. *Joenia*, *Trichonympha*, *Microjoenia*, *Monocercomonas*, *Dinenympha*, *Pyrsonympha*, and *Holomastigotes*, three of these being marked as new¹.

Three years prior to the appearance of the above work of Grassi and Sandias, Simmons (1890) described the organisms harboured by termites in Calcutta (genera and species not mentioned). He did not give names to the intestinal protozoa, and his descriptions are not sufficiently detailed for it to be possible now to determine their systematic position. His paper is of interest, however, as it constitutes the first record of the occurrence of protozoa in Asiatic termites. In 1891, Frenzel described a form referred to a new genus, *Leidyonella*, found in *Eutermes inquilinus* of the Argentine. A work by Porter, dealing with the forms previously studied by Leidy, appeared in 1897, and largely enriched our knowledge of their structure.

All the above-mentioned works were concerned with morphology alone, and no account of the mode of multiplication and development, based upon accurate observations, was given until 1904, when there appeared a description of the multiplication of *Joenia* by Grassi and Foà, and of *Trichonympha* by Foà. In the following year, Foà (1905) described two forms belonging to new genera, *Calonympha* and *Devescovina*. In 1910 a form wrongly referred to a new genus, *Lophophora*, was reported from Italy by Comes (*vide infra*), and Dobell also described a form from Ceylon, establishing a new genus *Gymnonympha* for it. In the same year appeared a work by Hartmann (1910), embodying the results of his studies on the organisms harboured by *Coptotermes hartmanni* of Brazil, and some theoretical considerations based upon his studies. He distinguished three forms and interpreted them as the young, the male, and the female of a species called *Trichonympha hertwigi*. With regard to the position of these peculiar organisms in the system of the protozoa, two different views prevailed at that time, some authors classifying them under the Mastigophora, while others placed them among the Ciliata. In the above-mentioned work, Hartmann attempted to establish a new division, Trichonymphida, independent of both the Mastigophora and the Ciliata. This work, however, was soon followed by a paper by Grassi and Foà (1911), pointing out that Hartmann's memoir contains many careless judgments and erroneous conceptions. They disapproved of Hartmann's view of the relationship of the three types which he described, and Grassi established two new genera, *Pseudotrichonympha* and *Holomastigotoides*, for the forms taken by Hartmann for "males" and "females" respectively ("male" and "female" misprinted in reverse in the original, and corrected afterwards). They did not, moreover, accept the view of Hartmann as regards the systematic position, and insisted upon their old classification of the organisms among the flagellates. In this

¹ Namely, *Joenia*, *Microjoenia*, and *Holomastigotes*. But it should be noted that the first of these was really named by Grassi in 1885, and the other two in 1892.

paper, also, they made revisions of the hitherto described genera and species, and established four new genera, besides the two above-mentioned, viz. *Eulophomonas*, *Mesojoenia*, *Spirotrichonympha*, *Microrhopalodina*.

Janicki published a beautiful work on *Lophomonas* in 1910. *Lophomonas* is the only known genus closely allied to the organisms under consideration which occurs in insects other than termites—it being found in the intestine of the cockroach. In two works which appeared in the following years (1911 and 1912), he dealt with the structure and multiplication of flagellates in the intestines of cockroaches and termites. The termites studied by him were two species of *Calotermes* (*C. castaneus* from Hawaii and *C. grassii* from Chile), and he established two new genera for their protozoa, viz., *Parajoenia* and *Stephanonympha*. In 1915 Janicki published a larger work, embodying the results of his further studies on the organisms harboured by the above two species of termites. In this paper he described seven forms, referred to the five genera *Devescovina*, *Parajoenia*, *Stephanonympha*, *Oxymonas*, *Foaina* (the last two being new), and several flagellates of uncertain genera, together with his studies on division, his views on the systematics of the group, and some other items.

During the years 1910–1913, Bugnion (partly with his co-workers, Popoff and Ferrière) published several papers dealing with the termites of Ceylon and the casts of termites generally. In these works, descriptions and figures of several intestinal protozoa were given, which are, however, not calculated to enrich our knowledge of these organisms. In 1912 appeared two works by Comes, dealing with the development of *Pyrsonympha* (a preliminary note for one of these (1910 *a*) had appeared two years before), and in 1914 another paper describing a species of “*Monocercomonas*” found in *Termes lucifugus* and *Calotermes flavicollis*. In all of the papers by Comes there are many mistakes in identification of genera and wrong understandings of several forms.

In 1915 Zulueta gave a description of nuclear division in a species of “*Dinenympha*” studied in Spain. Dogiel (1916) soon afterwards described four species of protozoa harboured by a *Rhinotermes* (?) from Uganda, referring them to three genera, viz. *Trichomonas* (*Tetratrachomonas*), *Gigantomonas*, and *Myxomonas*, the latter two being newly established. In 1916 also appeared a work by França, dealing with organisms referred to two genera, *Trichonympha* and *Leidya* (gen. nov.), in the *Leucotermes* of Portugal; and three more papers (França, 1918, 1918 *a*, 1918 *b*) were published later, which embodied the results of his further studies and his opinions on the classification of these forms. The two genera (*Leidya* and *Caduceia*) established by him, however, are probably synonyms of *Spirotrichonympha* and *Devescovina* respectively.

In 1917 appeared a large work by Grassi, which contains the descriptions of many forms found in *Porotermes adamsoni* from Australia, *Epicalotermes aethiopicus* from Eritrea, *Coptotermes sjöstedti* from French Guinea, *Copto-*

termes lacteus from Australia, *Schedorhinotermes putorius* from Australia, and *Schedorhinotermes intermedius* from French Guinea. He referred the protozoa to 13 genera, 18 species, and 5 varieties—5 of the 13 genera, viz. *Joenia*, *Staurojoenia*, *Spirotrichonymphella*, *Pseudotrypanosoma*, and *Diplonympha*, being newly established ones.

In a work on the structure and biology of *Archotermopsis* of India, published in 1919, Imms gave descriptions of the intestinal protozoa harboured by that termite. Further studies of these organisms are being carried on by Cutler, who obtained a stock of the living termites from Imms, and two papers (1919 and 1920) have already been published, embodying his studies on species of *Trichomonas* (*Ditrichomonas*) and *Joenopsis* (gen. nov.). Kofoid and Swezy published in 1919 a series of papers dealing with the structure and mode of multiplication of four species of intestinal protozoa harboured by *Termopsis angusticollis* of California. Each species was referred to a different genus, viz. *Streblomastix*, *Trichomitus*, *Trichonympha* and *Leidyopsis*, the first and the last being new.

The physiological relationship between the termites and their intestinal protozoa is a highly important and deeply interesting subject. Do the organisms live in the intestine parasitically, commensally, or symbiotically, as different authors have supposed? A study of this question has been made by Buscalioni and Comes (1910). They examined the reactions to chemical reagents of each region of the body of the protozoa, to determine the fate of the ingested wood fibres, and reported their results in detail. Imms (1919) has also devoted one chapter of his work to the consideration of some physiological questions.

A note by Buttel-Reepen (1914) recording the results of his examination of many genera of termites in Java, Sumatra, Malacca, and Ceylon, to determine the presence or absence of intestinal protozoa, will be suggestive for further studies; but his work, though adding to our knowledge of the distribution of these protozoa, contains nothing else of interest for the present paper.

A list of genera hitherto described will be given here. The genera established for the organisms harboured exclusively by termites, the type species of these genera, and their synonyms, are as follows:

GENUS.	TYPE SPECIES.
<i>Trichonympha</i> Leidy, 1877.	<i>T. agilis</i> Leidy.
<i>Pyrsonympha</i> Leidy, 1877.	<i>P. vertens</i> Leidy.
<i>Dinenympha</i> Leidy, 1877.	<i>D. gracilis</i> Leidy.
<i>Joenia</i> Grassi, 1885.	<i>J. annectens</i> Grassi.
<i>Leidyonella</i> Frenzel, 1891.	<i>L. cordubensis</i> Frenzel.
<i>Microjoenia</i> Grassi, 1892.	<i>M. hexamitoides</i> Grassi.
<i>Holomastigotes</i> Grassi, 1892.	<i>H. elongatum</i> Grassi.
<i>Devescovina</i> Foà, 1905.	<i>D. striata</i> Foà.
<i>Calonympha</i> Foà, 1905.	<i>C. grassii</i> Foà.
<i>Gymnonympha</i> Dobell, 1910.	<i>G. zeylanica</i> Dobell.

GENUS.	TYPE SPECIES.
<i>Lophophora</i> Comes, 1910. = <i>Pyrsonympha</i> Leidy.	<i>L. vacuolata</i> Comes.
<i>Microrhopalodina</i> Grassi, 1911.	<i>M. enflata</i> Grassi.
<i>Eulophomonas</i> Grassi, 1911.	<i>E. calotermitis</i> Grassi.
<i>Mesojoenia</i> Grassi, 1911.	<i>M. decipiens</i> Grassi.
<i>Pseudotrichonympha</i> Grassi, 1911.	<i>P. hertwigi</i> (Hartmann) Grassi.
<i>Spirotrichonympha</i> Grassi, 1911.	<i>S. flagellata</i> Grassi, 1893.
<i>Holomastigotoides</i> Grassi, 1911.	<i>H. hertwigi</i> (Hartmann) Grassi.
<i>Parajoenia</i> Janicki, 1911.	<i>P. grassii</i> Janicki.
<i>Stephanonympha</i> Janicki, 1911.	<i>S. silvestrii</i> Janicki.
<i>Oxymonas</i> Janicki, 1911.	<i>O. granulosa</i> Janicki.
<i>Foaina</i> Janicki, 1911.	<i>F. gracilis</i> Janicki.
<i>Leidya</i> França, 1916. = <i>Spirotrichonympha</i> Grassi.	<i>L. metchnikovi</i> França.
<i>Gigantomonas</i> Dogiel, 1916.	<i>G. herculea</i> Dogiel.
<i>Myxomonas</i> Dogiel, 1916.	<i>M. polymorpha</i> Dogiel.
<i>Joenia</i> Grassi, 1917.	<i>J. pulchella</i> Grassi.
<i>Staurojoenia</i> Grassi, 1917.	<i>S. mirabilis</i> Grassi.
<i>Spirotrichonymphella</i> Grassi, 1917.	<i>S. pudibunda</i> Grassi.
<i>Macrotrichomonas</i> Grassi, 1917.	<i>M. pulchra</i> Grassi.
<i>Pseudotrypanosoma</i> Grassi, 1917.	<i>Ps. giganteum</i> Grassi.
<i>Diplonympha</i> Grassi, 1917.	<i>D. foae</i> Grassi.
<i>Caduceia</i> França, 1918. = <i>Devescovina</i> Foà.	<i>C. theobromae</i> França.
<i>Streblomastix</i> Kofoid et Swezy, 1919.	<i>S. strix</i> Kofoid et Swezy.
<i>Leidyopsis</i> Kofoid et Swezy, 1919.	<i>L. sphaerica</i> Kofoid et Swezy.
<i>Joenopsis</i> Cutler, 1920.	<i>J. polytricha</i> Cutler.

Other genera of protozoa of which species have been reported in the intestines of termites are the following:

Flagellata	{	<i>Monocercomonas</i> [<i>M. termitis</i> Grassi].
	{	<i>Trichomonas</i> (<i>Ditrichomonas</i>) [<i>T. (D.) termitis</i> (Imms) Cutler].
	{	„ (<i>Tetratrichomonas</i>) [<i>T. (D.) termitidis</i> (sic) and <i>macrostoma</i> Dogiel].
	{	<i>Trichomitus</i> [<i>T. termitidis</i> (sic) Kofoid et Swezy].
Ciliata	<i>Nyctotherus</i> [<i>N. termitis</i> Dobell].
Sporozoa	...	“ <i>Gregarina</i> ” [“ <i>G.</i> ” <i>termitis</i> Leidy].

2. MATERIAL AND METHODS.

The species of termites recorded as occurring in Japan and its territories are rather large in number (16 species according to M. Oshima, and 13 or 15 species according to Holmgren and Hozawa). As a matter of fact, the termite fauna is remarkably rich in the subtropical and tropical regions, so that all of the reported species are found in Formosa¹, while in Japan proper²

¹ The island of Formosa lies at the southern extremity of the Empire of Japan, extending from 120° 2' to 122° 6' E. of Greenwich, and in latitude from 21° 45' to 25° 37' N.; the area covering 13,908 square miles.

² Conventionally we divide the Empire into Japan proper, Formosa, Saghalien, and Chosen (Korea)—Chosen being a peninsula of the continent, Saghalien an island at the northern extremity, and Japan proper the other islands forming the main portion of the Empire.

there occur only two species, viz. *Leucotermes* (*Reticulitermes*) *speratus* and *Coptotermes formosanus*. Though the termites of Formosa are so rich in species, the great majority of these species are not of common occurrence, but are rare or locally distributed, and only a few species are found commonly throughout the island. The species most commonly found in Formosa are *Coptotermes formosanus* and *Odontotermes formosanus*. A species of *Leucotermes* is also more or less frequently found in Formosa, and referred to different species by different authors. M. Oshima distinguished it from *Leucotermes speratus*, saying that although it is scarcely possible to draw a sharp line of distinction between the imagines, yet the soldiers display some distinct differences which justify their separation. S. Yano, S. Hozawa, and Holmgren, on the other hand, persist in the belief that they should be united into one. Not being an entomologist, I myself will not express an opinion on this species question. As regards their protozoa, however, I find that distinct differences are recognizable between those of *Leucotermes speratus* of Japan proper and those of the disputed species of Formosa. Thus I feel it convenient for my description to let the Formosan species have its own name, and I prefer to call it by the name given by M. Oshima, *Leucotermes* (*Reticulitermes*) *flaviceps*.

In carrying out my studies both in Tokyo and Formosa, I was fortunate in obtaining an ample supply of living specimens of four species of the host insects. While at Tokyo, my studies were done on the forms harboured by *Leucotermes speratus*, collected at several localities in Japan proper. On going afterwards to Formosa, three different species, *Coptotermes formosanus*, *Leucotermes flaviceps*, and *Odontotermes formosanus*, came into my hands. Moreover, I have had an opportunity of examining another species of rather rare occurrence, *Capritermes nitobei*. In *Odontotermes formosanus* and *Capritermes nitobei*, however, none of the protozoa under discussion were found. While working at Tokyo, I was unable to obtain any specimens of *Coptotermes formosanus*; but shortly before finishing this study I made a trip to Kyushu, for the purpose of studying *Coptotermes formosanus* and *Leucotermes speratus* in that southern district of Japan proper.

In all the three species of termites studied by me (*Leucotermes speratus*, *L. flaviceps*, and *Coptotermes formosanus*), the protozoa are found in equally large numbers, and almost the entire cavity of the intestine of every individual is found filled with them, excepting the oesophagus and the proventriculus. The Malpighian tubules also were found frequently invaded by some of the forms with slender bodies. Young termites, lately hatched, have their intestines free from protozoa; but as they begin to take food, the protozoa seem quickly to invade them, for individuals measuring only three millimeters in length are usually found rather heavily infected.

In the workers the intestine is more developed than in the other casts, and is found almost filled with protozoa, food substances being rather small in volume as compared with the contained organisms. In the soldier and

the nymph the intestine is rather slender, but is also almost filled with protozoa. In the imago the condition is variable; in some individuals the intestine is quite free from protozoa while in others they are present in rather large numbers. Regarding the infections of imagines, my experience is not sufficiently extensive, and further investigations are needed.

The protozoa found in every individual termite are abundant, not only in number, but also in species. Almost all the kinds proper to a particular species of host are commonly found in every individual of that species. No differences are recognizable among the protozoa found in workers, soldiers, and nymphs, and all kinds usually occur with equal frequency in the above casts. In imagines which contain protozoa, the conditions seem also identical.

The fact that the protozoa are present with such frequency is probably to be explained by the habits of the termites themselves. They live in communities, and, as is well known, eat one another's faeces. The space in which they live is usually dark and moist, so that the faeces must usually remain damp, and the protozoa passed out with them, even though not encysted, may thus remain alive for some time. It seems probable also that the same habits explain the fact that the young termites become heavily infested in very early stages of their development.

As mentioned in the foregoing historical review, the physiological relationship between these protozoa and their insect hosts is a subject of profound interest and great importance. For the consideration of the rôle played by the protozoa in the digestion of wood fibres in the intestine, the habits of all termites, both those harbouring protozoa and those free from them, must be carefully investigated.

I may add here a brief note on the habits of the species studied by myself. *Leucotermes speratus* and *L. flaviceps*, harbouring *Trichonympha*, *Teratonympha*, *Holomastigotes*, *Microspironympha*, and *Pyrsonympha*, are species doing some damage to wooden structures. These species form irregular cavities and burrows under or in rotten boards, timbers, decayed logs, fallen trees, and similar material. *Coptotermes formosanus*, harbouring *Pseudotrichonympha*, *Holomastigotoides*, and *Spirotrichonympha*, is the most destructive species in Japan, especially in Formosa. Wooden buildings, furniture, logs, and decayed portions of living trees are severely attacked by this species. *Odontotermes formosanus*, harbouring none of these protozoa, sometimes attacks also timber and trees, though not causing such extensive damage. Soft materials, however, such as the stems and roots of sugar cane are often destroyed by this species. *Capritermes nitobei* in which I did not find any of the protozoa in question—though my material was not abundant—has similar habits to those of *Odontotermes*.

The fluid medium chiefly used for the observation of living organisms was a 0.3–0.4 per cent. solution of sodium chloride. The same fluid was also used for diluting the gut contents in the preparation of fixed smears. Observations of the living organisms were found most important in studying these forms;

and several minute points of structure, which can hardly be made out in fixed and stained preparations, are clearly observable in fresh specimens. Dark-ground illumination and vital staining methods were tried and proved very fruitful in many cases. For the fixative, Schaudinn's solution was almost exclusively used, and the smears, prepared on cover-glasses, were fixed by dropping them face downwards on the fixative. The most excellent and frequently used stains were iron-haematoxylin methods. I used in most cases 2 per cent. solution of iron-alum and ripe alcoholic solution of haematoxylin. Giemsa's fluid, thionin, fuchsin, and other aniline dyes were also used and found useful in several cases. Some bacteriological stains were also used for the study of special structures. Preparations fixed and cut into sections were also made, but were found unsuitable for morphological study; and my experience taught me that this method is apt to lead to erroneous judgments. Thus total preparations were chiefly relied upon, except for the purpose of studying the position of the protozoa in the intestine and their relation to its wall.

3. DESCRIPTION OF FORMS FOUND IN JAPANESE TERMITES.

The present paper embodies the results of my studies of forms referred to eight genera. Six of these eight are genera known already to occur exclusively in the intestines of termites; the other two are new, and belong to the same group. Other protozoa were also found in the termites studied. These forms are of smaller size, and while some are trichomonad types, others are of peculiar organization. My studies of these forms are, however, not complete, so that I shall refrain in this paper from making any further mention of them.

Before entering into a description of each form, a brief synopsis of the genera and species will be given here, as I think it will be helpful to the reader.

SYNOPSIS OF GENERA AND SPECIES.

Our forms are conveniently divided into two main groups, which may be called (1) the *Trichonympha* series and (2) the *Pyrsonympha* series. These groups are not to be taken as constituting families or higher orders: they are used here merely for convenience in description. The classification of the organisms contained in them will be discussed later (p. 300). The first series contains forms provided with a very large number of flagella, and the second those with only four or eight flagellar cords. The species belonging to the former series are found in both *Coptotermes* and *Leucotermes*, while those of the latter are limited to *Leucotermes*.

(1) *Trichonympha* series.

Six genera are placed in this series. It may be subdivided into two groups, of which *Trichonympha* and *Holomastigotoides* may be regarded as the representative types.

A. *Trichonympha* group.

Large forms of complicated organization, provided with numerous flagella. Three genera are distinguished:

I. *Trichonympha* Leidy.

Body oval or lanceolate, its posterior region destitute of flagella. I distinguish two varieties of a single species:

- (a) *T. agilis* var. *japonica* var. nov.¹ (in *Leucotermes speratus*);
- (b) *T. agilis* var. *formosana* var. nov. (in *Leucotermes flaviceps*).

II. *Pseudotriconympha* Grassi.

Very large, and spindle-shaped; the posterior region of the body provided with flagella arranged in spiral rows. One species:

P. grassii sp. nov. (in *Coptotermes formosanus*).

III. *Teratonympha* gen. nov.

Very large, long and club-shaped. Numerous transverse ridges on the surface give the organism a segmented appearance, so that it resembles a cestode. Flagella arranged in transverse rows. One species and one variety:

- (a) *T. mirabilis* gen. nov. et sp. nov. (in *Leucotermes speratus*);
- (b) *T. mirabilis* var. *formosana* var. nov. (in *Leucotermes flaviceps*).

B. *Holomastigotoides* group.

Large or small forms, less complicated than the preceding. Flagella arising in rows, which have a common point of origin at the anterior extremity and wind spirally backwards. Four genera are distinguished:

IV. *Microspironympha* gen. nov.

Body small, and spindle-shaped or piriform. A tubular structure connects the anterior tip of the body and the nucleus. Flagellar bands few, a rather large portion of the posterior region being free from them. One species:

M. porteri gen. nov. et sp. nov. (in *Leucotermes flaviceps*).

V. *Holomastigotoides* Grassi.

Very large, piriform or ovoid, with flagellar bands distributed densely over almost the entire surface of the body. One species:

H. hartmanni sp. nov. (in *Coptotermes formosanus*).

¹ Throughout this paper I have designated the new forms which I have found in Japanese termites as "var. nov.," "sp. nov.," etc. The majority of these have, however, already been described and named in my earlier papers (1916, 1917). But these were written in Japanese, and were therefore inaccessible to most zoologists; and I am, moreover, uncertain whether the names can be regarded as having any status in international nomenclature. I have therefore marked all such names as technically "new" in the present memoir.

VI. *Spirotrichonympha* Grassi.

Body conical and rather small. Flagellar bands fewer, distributed over almost the whole of the body. The bands are peculiar in their disposition, being situated somewhat deeply in the protoplasm. One species:

S. leidy sp. nov. (in *Coptotermes formosanus*).

VII. *Holomastigotes* Grassi.

Body small and spindle-shaped, its surface spirally ridged; flagella arising in the grooves behind the ridges. One species:

H. elongatum Grassi (?) (in *Leucotermes speratus* and *L. flaviceps*).

(2) *Pyrsonympha* series.

The body large or small, and club-shaped, fusiform, or piriform. An axial filament, fixed anteriorly, runs down the body. Four or eight slender cords start at the tip of the filament and run spirally backwards, fixed on the body wall, to the posterior extremity, where they emerge as free flagella.

A single genus, *Pyrsonympha*, divided into two subgenera, *Pyrsonympha* and *Dinenympha*. A synopsis of the subgenera and the species will be given at the beginning of the description of these forms (p. 281).

(1) *Trichonympha* Series.I. *Trichonympha*.

This is one of the three oldest genera established in 1877 by Leidy for the American species. Under this genus six species have hitherto been described, viz. *T. agilis*, *T. leidy*, *T. minor*, *T. magna*, *T. hertwigi*, and *T. campanula*. *T. agilis* is the species described by Leidy (1877 and 1881) in America, and its behaviour and structure, as far as they could be studied in the living condition, were carefully observed and accurately described and figured by him. Some 20 years later, Porter's work on the same species appeared (1897), and threw much new light upon the finer structures. This species was also found in Italy, and was described by Grassi¹ in his famous work with Sandias (1893) on the termites. At first the Italian forms were considered to be identical with the American, but afterwards two types, *major* and *minor*, were distinguished by Foà (1904). The latter type was subsequently made by Grassi (1911) into a species, *T. minor*. In recent years several reports dealing with the forms belonging to this genus have been published. França (1916 and 1918) described the structure of an organism, identified as *T. agilis* by him, harboured by Portuguese termites. In 1917 a voluminous paper by Grassi appeared, in which large numbers of species of many genera, harboured by termites from various localities, were dealt with; and one new species of this genus—*T. magna*—was described from an Australian termite. One more report was published by Kofoed and Swezy in 1919. They described a new

¹ According to Grassi (1885) the organism was discovered in Italy by Condorelli.

species, *T. campanula*, harboured by *Termopsis* in North America, and its structure and mode of multiplication were discoursed in detail.

In papers which appeared in earlier years, the generic name *Trichonympha* was erroneously applied to various forms with quite different characteristics. For instance, a form briefly described by Kent (1885), shortly after the appearance of Leidy's work, and named *T. leidy*, is doubtless a member of another genus. More recently, this generic name was applied by Hartmann (1910) to the organisms—"T." *hertwigi*—harboured by Brazilian termites. But these present peculiarities proper to more than two other genera, and Grassi soon established two new genera for them.

Forms belonging to this genus are found in *Leucotermes* of both Japan proper and Formosa. Our forms show a rather close resemblance to *T. agilis*, described by the American authors, but some points of difference are definitely recognizable among them. There are, moreover, some distinct differences between those of *Leucotermes speratus* of Japan proper and those of *Leucotermes flaviceps* of Formosa. The differences found among the three forms, however, are not judged to be sufficiently great to make them separate species, and I propose to treat our forms as varieties of the American species, calling them *T. agilis* var. *japonica* and var. *formosana*.

(1) *Trichonympha agilis* var. *japonica* var. nov. (Plate X, figs. 1-4).

The body assumes an oval shape, slightly narrowed at the anterior end and evenly rounded at the posterior. The dimensions are usually 70-90 μ in length, and 40-70 μ in breadth. The body is clearly differentiated into anterior and posterior regions ("head" and "body" according to Leidy's terminology). The front end of the former is drawn out into a nipple-like prominence. The anterior region, occupying some one-fourth of the whole length, is quite different in structure from the rest of the body; its wall being remarkably thick, and its endoplasm seeming denser, so that no marked changes in shape are observed in this region. In the posterior region, on the other hand, the body wall is not so thick and the endoplasm appears less dense, so that it is rather metabolic. Porter called the anterior eminence the "nipple" and the rest of the "head" the "bell-shaped region," or simply the "bell." The terms "nipple" and "bell" will be used in this paper. In *Trichonympha agilis*, the anterior region is of about the same length as the posterior, or a little shorter; that is to say the bell is markedly larger than that of our variety. In *T. campanula* the bell is still larger.

One of the conspicuous features of the forms of this genus is the coat of flagella, thickly distributed on the anterior region. They are very closely set on both the nipple and the bell, excepting only the specially differentiated anterior tip. These flagella are directed backwards and increase in length towards the boundary of the anterior and the posterior regions. The longest ones attain so remarkable a length as to extend beyond the posterior extremity

of the body. The flagella are directed backwards, applied more or less closely on the body wall, and are regularly arranged, so that they give the surface of the body a very beautiful appearance of parallel stripes.

The nipple is a very complicated structure (Pl. X, fig. 13). It has the shape of a cone with an evenly rounded top, and rests on the anterior border of the bell. Its height is about 10μ and its width is about two-thirds of the height. It is remarkably rigid in consistency, so that it hardly shows any change of shape, even when the anterior portion of the body is swung actively in all directions. Four parts can be distinguished in the nipple, which were named by Porter as follows: (1) the axial rod; (2) the inner layer; and (3) the outer layer, surrounding the axial rod; and (4) the "cap" at the top of the nipple. I propose to follow the above terminology, altering only "axial rod" into "axial core." Of the above four parts, the axial core and the inner layer come together so as to form the axis of the nipple. The inner layer encircles the greater part of the axial core, leaving uncovered only a small portion of the apical part, which expands into a knob-like enlargement. The columnar part of the axial core is of almost uniform width, but slightly narrowed towards the anterior end, and having a constriction behind the knob. The knob and the constricted portion are quite structureless and refractive; while the columnar part is not homogeneous, but consists of two parts, namely, an outer wall of almost uniform thickness, and its contents—the latter appearing slightly granular and being continuous behind with the endoplasm of the bell. The inner layer is homogeneous in structure and refringent. The outer layer covers the inner layer throughout its whole extent. It is widest at the base, and is there about twice as thick as the inner layer. It gradually decreases in thickness towards the front end, where it becomes very much tapered and terminates under the knob of the axial core, together with the inner layer. Thus the above three parts assume anteriorly, as a whole, the shape of a dome with a knob resting on its top.

The "cap" is a structure with the shape of a hemispherical bowl: it occupies the top of the nipple, with its margin resting on the sloping surface of the outer layer. The space confined by the cap in front of the above described dome-shaped part is filled with a homogeneous substance. This part of the nipple is very rigid in this variety, and displays no recognizable alterations of shape. The outer layer is traversed by the fine flagella, which reach as far as the outer surface of the inner layer. The flagella are so numerous and densely set, that, both in top view as well as in side view, they appear to occupy the greater part of the outer layer. The most anterior flagella pass downwards along the sloped surface of the frontal part of this layer, and become free at the place where the margin of the cap rests upon it. The flagella are not of uniform length. Those situated at the tip are the shortest, and are nearly as long as the nipple. They gradually increase in length towards the base of the nipple, where they become twice as long as the height of the latter.

The knob and the narrow portion of the axial core stain intensely with iron-haematoxylin, as well as with eosin. The wall of the columnar part also stains deeply, while its contents do not take any dyes at all, but contain deeply stained granules. The inner layer stains only slightly or not at all, while the outer layer appears rather deeply stained. In well-stained preparations further differentiation, which has been overlooked by previous authors, is discernible in the inner layer. This consists of a zone, somewhat darkly stained, close to the axial core, and measuring some one-fourth of the layer in its thickness. The boundary between the inner and the outer layers stains intensely with haematoxylin, thionin, fuchsin, and other dyes. The existence of such a zone, and the structure of the axial core and the inner layer, afford us, together with the result of studies of living specimens, strong evidence for believing that the flagella arise from this zone.

It has already been remarked that the bell is distinctly differentiated from the rest of the body, especially as regards its wall. The wall is very thick and one can easily detect the outer and the inner layers both in the living animal and in stained preparations. These layers are of the same structure as the corresponding layers enclosing the axial core, and are apparently continuous with them. There is, however, as pointed out by previous authors, a circular fissure ("citartrosi" of Grassi) encircling the base of the nipple and reaching the base of the axial core. It is, therefore, by means of the axial core only that the nipple is connected with the body. As in the nipple, the outer layer of the bell is much thicker and stains more deeply than the inner layer. The latter stains feebly or not at all, excepting a zone at the base, which stains more deeply like the corresponding zone in the nipple. In the living animal, the inner layer at once attracts the observer's attention as a transparent zone. In stained preparations the deeply stained zone at the boundary between the two layers is much more conspicuous than in the nipple. The numerous long flagella undoubtedly arise from this zone and traverse the outer layer.

The descriptions of the structure of the nipple and the wall of the bell in *Trichonympha agilis*, given by previous authors, do not agree with the results of my observations in several points. As for the axial core, to begin with, the descriptions of Porter (1897) almost agree with mine, except that he describes a slight difference in its shape. Grassi's descriptions seem mostly to agree with mine, but differ in the following point. According to him, the anterior knob is not solid, but consists of two parts, namely, wall and contents, just as in the columnar part; the contents of both parts being the same, and continuous with each other. The axial core of the Portuguese species, described by França, is of a quite peculiar structure. He described (1916) and illustrated (1918) this structure as consisting of a mushroom-shaped body and a projecting transverse disc, connected with each other by a commissure. He did not mention any differentiation of its wall and contents. According to Kofoid and Swezy (1919), the structure of the nipple is very peculiar in *T. campanula*. There is no deeply stainable tubular wall, but several longi-

tudinal strands of deeply stainable substance are observed surrounding the columnar core, corresponding to the contents of the axial core in *T. agilis*. At the anterior end, there is no structure corresponding to the knob in *T. agilis*; and there is either only a ring of deeply stainable substance at the tip, or else the strands are simply united together at the anterior end. The descriptions of the layers surrounding the axial core, of the wall of the bell, and of the flagella, given by previous authors, are also diversified. According to Porter, the inner layer of the bell is marked with fine lines perpendicular to the surface of the body wall, and he took these lines to be traces of traversing flagella.

Grassi's statements are, to me, very confusing. He describes several different appearances seen by him, and does not give any interpretations to reconcile his diverse observations. His opinion seems, however, to be as follows. The inner layer is liquid, and the basal granules of the flagella are found at the base of this layer, situated on longitudinal ridges lying directly under it. In some of his figures, the ridges are shown as forming a distinct layer. According to him, the flagella are sometimes provided with a node or granule at the outer boundary of the layer. França described the flagella as arising from the surface of the axial core; those of the nipple from the mushroom-shaped body and those of the bell from the disk-like part. A differentiation into layers, both in the nipple and in the bell, seems not to be recognized by him; but the existence of such a differentiation cannot be doubted. The body wall of *T. campanula* is figured by Kofoid and Swezy (1919) in various ways and their descriptions are not easy to understand; but, according to Professor Kofoid's personal explanation—which he kindly gave me in London—the wall of the bell consists of four layers. The flagella arise at the outer boundary of the innermost layer (drawn homogeneous in some figures and granular in others), and each flagellum has a node or granule at the middle of the portion embedded in the body wall. The outer part of the body wall, traversed by the flagella, is divided by an alveolar layer into outer and middle layers.

There are thus many points of disagreement among the statements of previous authors, and between theirs and mine; and I cannot but doubt the accuracy of their observations. It will be worthy of mention, here, that some of the above views were founded upon observations made on material fixed and cut into sections, which I believe, from my own experience, are apt to lead us to erroneous results. The space encircling the base of the nipple is described by Grassi, and Kofoid and Swezy, as rather spacious. But it is usually a narrow slit in my organisms, and it was only in specimens badly fixed or cut into sections that I found it wide and distinct.

In the posterior region, the body wall is not thick, though it is fairly rigid and stains rather deeply. No clear layer corresponding to the inner layer of the anterior region is here recognizable.

The endoplasm is finely granular, homogeneous, and appears slightly grey

in colour during life. Beneath the body wall is seen a zone of particularly coarse and deeply stainable granules. It is specially dense under the thick wall of the bell. This layer of granules lies beneath the body wall, and it is found in good preparations that they are embedded in a very thin layer of transparent and structureless substance lying immediately under the body wall.

The nucleus is invariably situated in the median line and a little anterior to the middle point. It does not lie freely in the endoplasm, but has a special structure keeping it in connexion with the body wall. This interesting structure was called by Grassi the "cestello" or "little basket," and I propose to use the term "corbule" for it in this paper. The corbule has the following structure. In living as well as in stained preparations, a distinct zone of granules, identical with those distributed beneath the body wall, is seen on both sides of the nucleus, connecting its posterior surface with the wall of the bell. Careful study reveals the presence of a thin membranous structure resembling an inverted cone or a hemispherical bowl, with the granules on its internal surface; and it can be seen that the nucleus is situated at its bottom. At the edge of the bell the granules lining the membrane pass gradually over to the well-developed layer of similar granules under the body wall. In the peripheral part of this structure, the granules are distributed as deeply as under the wall of the bell; and the outline of the structure itself is so sharp on its external surface that the existence of a distinct membrane seems quite certain. Towards the nucleus, however, the membrane becomes gradually indistinct, and it is often quite obscure in the immediate vicinity of the nucleus. The granules are commonly distributed uniformly, both under the wall of the bell and on the membrane, but in some individuals a regular alternate arrangement of dense and thin bands of granules is noticeable (Pl. X, fig. 4). The bands are usually of almost uniform width and are found, in well-developed cases, extending from the middle part of the bell to the vicinity of the nucleus.

The shape of the corbule is more or less variable. Usually it assumes, in my organisms, the shape of a hemispherical bowl, with the nucleus situated just at its bottom; neither the membrane nor any trace of the granules being discernible behind the nucleus (Pl. X, fig. 1). In some individuals, however, it assumes the shape of an inverted cone, touching the nucleus tangentially; and the membrane is then visible behind the nucleus, with granules attached to it, and sometimes appears fibrous (Pl. X, figs. 2 and 3). In some other individuals the structure is indistinct in the vicinity of the nucleus, and an irregular mass of granules is seen around the latter. In my organisms still another structure, keeping the nucleus in connexion with another part of the body, is recognizable. This is a columnar mass of somewhat indistinct contour, extending perpendicularly from the apex of the bell to the nucleus (Pl. X, fig. 3). It apparently consists of the same kind of granules as those under the wall of the bell and on the corbule, but I am of the opinion that there is really

a matrix of special protoplasm—which I think to be the same as that of the corbule—in which the granules are embedded. The columnar structure is commonly thick and distinct at the anterior part and becomes gradually indistinct towards the nucleus. In some individuals the columnar structure can be traced clearly to the anterior surface of the nucleus, but appears in some others to end before reaching it. The endoplasm in front of and behind the corbule is not continuous. In the posterior part it is less dense and contains various kinds of food débris.

In the American species Leidy distinguished the “head endosarc” from the “body endosarc,” but the existence of a membranous structure (corbule) at the boundary was overlooked by him. Porter accurately described a “coarsely granular protoplasmic partition,” but he also did not recognize the existence of a membrane. Grassi distinguished the corbule (“cestello”) in the Italian form, and described it as consisting of numerous rodlets (“bastoncelli”). As described above, the corbule shows no such fibrous or rodlike structure in my organisms. The zonal arrangement of thin and dense areas of granules, observed very often in my organisms, does not suggest, to me, the existence of fibres. I conjecture the zonal appearance to be due to thickenings of the substance of the membrane, or a zonal arrangement of the granules, in consequence of contraction of the body or such other causes. Grassi conjectured the membrane to be continuous with the inner layer of the body wall, but I do not think this is so; and I am of the opinion that a layer of the same substance as the membrane may exist under the wall of the bell, keeping the corbule in connexion with the axial core of the nipple. I believe also that the columnar mass hanging from the base of the nipple to the nucleus, and the contents of the axial core, are composed of the same substance. A comparative study of similar structures in allied forms seems to afford us some basis for such conjectures. França described the corbule as an ellipsoidal sac hanging from the base of the nipple. I think his statement may be true, but undoubtedly the thick body wall of the bell, overlooked by him, and the upper half of his “cestello,” is nothing but the basal line of the wall of the bell.

According to Kofoed and Swezy (1919) no structure corresponding to the corbule is noticeable in *T. campanula*, and there is a complicated system of fibres and myonemes, called by them the “neuromotor system.” They describe the neuromotor system as consisting of oblique fibres, longitudinal ridges with the basal granules of the flagella, longitudinal myonemes, and transverse myonemes; the first three being continuations of the axial core of the nipple (“centroblepharoplast” in their terminology). As far as my experience goes, there are no such fibres or myonemes recognizable in my organisms. As for the corbule in *T. campanula*, I conjecture that a similar but less differentiated structure may be present.

The nucleus is oval ($10\text{--}18\mu \times 8\text{--}12\mu$), and is provided with a thin but rigid membrane. It consists of a structureless ground substance and a variable

number of chromatin masses, which are found assembled at the centre, forming a large spherical heap surrounded by a clear zone. In the majority of cases, the chromatin masses are distinct in their outline and separated from each other. Sometimes, however, they are not so sharply contoured and distinctly isolated, but loosely connected to form a somewhat reticular mass. The chromatin masses in any one nucleus are of nearly uniform size; but in some individuals they are small in size and numerous, and in others few but large.

Despite my long search, carried out through all seasons, I have been able to find only a small number of dividing individuals. The observations of American authors agree with mine in this point. In the Formosan variety, on the other hand, dividing forms are found not infrequently, and I have had ample material for the study of the process of division, which seems to be identical with that of the variety under consideration. Thus no separate account of the process in the variety *japonica* will be given.

No forms which suggest the occurrence of other modes of development have been observed by myself. Leidy has figured several forms which he thought to be the young, but a contrary view was afterwards put forward by Porter. Some of the forms considered by Leidy to be the young are really met with in *Leucotermes speratus* in association with the present variety. But there is strong evidence indicating that they have nothing to do with *Trichonympha*, as pointed out by Porter, and have to be classified under different genera. The form which was taken by Porter—though with much misgiving—to “present the best evidence of being the young” is not found in *Leucotermes speratus*, though it is frequently met with in *Leucotermes flaviceps* accompanied by the other variety of *Trichonympha*. It is thus quite certain that there is no genetic connexion between Porter’s “young form” and the variety now under consideration.

(2) *Trichonympha agilis* var. *formosana* var. nov. (Plate X, figs. 5–12).

This variety is distinguished from the foregoing by its shape, the proportional dimensions of various parts of the body, and by the structure of some parts.

The difference in shape of the two varieties is recognized at a glance (compare Figs. 1 and 5, Plate X). The body length is nearly the same in the two varieties, while the breadth of *formosana* measures only a little more than one-half that of *japonica*. The organism assumes a spindle or a lanceolate shape, in contradistinction to the oval shape of the former variety, and the inclination of the wall of the bell is conspicuously acute. The head (including the nipple and the bell) occupies some one-sixth of the length; and it is thus a little smaller than that of the variety *japonica*. Though the body is slender in this variety, as described above, the nucleus is about the same size as in *japonica*, so that a larger portion of the breadth of the body is occupied by it in this form. Some differences are also recognizable in the nipple and the

wall of the bell. The axial rod of the nipple is devoid of the constriction, behind the anterior knob, seen in the former variety. The inner layer of the wall of the bell is thicker than in *japonica*. The deeply stained zone at the boundary of the inner and the outer layer of the bell is distinct and shows an appearance of a sheet of minute granules closely aggregated. The acuteness of the inclination of the wall of the bell and the proportionally large dimensions of the nucleus are associated with a difference in the shape of the membranous wall of the corbule. In the majority of cases, it assumes the shape of an inverted cone, or sometimes of a cylinder, touching the nucleus tangentially in the vicinity of its equator and stretching behind it.

Dividing forms are occasionally met with in this variety. In the termites taken directly from their nest, or those kept in large vessels under conditions supposed to be natural for them, dividing forms are very seldom met with; and a large number of termites have to be examined before hitting upon any dividing forms. I happened, however, to come across a method of getting dividing forms with less difficulty: namely, by keeping the termites, for several days, in a small glass dish with a small quantity of mud, and a piece of cotton-wool to serve as food. Such conditions acting upon the host seem to induce the multiplication of the intestinal organisms. It is rather remarkable that a large number of dividing forms may be found in a single host, while not a single one is met with in another subjected to the same conditions.

The division processes of the Italian species have been described by Foà (1904), and Kofoed and Swezy (1919) have described those of *T. campanula*. The division processes of the forms under consideration are of the same type as those described by these authors.

Actual division takes place at first in the nipple. It is bisected longitudinally into two equal halves, and a rather thick strand ("fuso esterno" of Foà, and "paradesmose" of Kofoed and Swezy) makes its appearance at the base of the daughter nipples, keeping them in connexion. This strand is somewhat fibrous and is stained rather feebly by iron-haematoxylin. Before the above-mentioned division takes place, nuclear changes usually occur. In the resting stage the nucleus is of the same structure as that of the former variety; but in the variety *formosana* the chromatin masses are more often not distinctly separated from one another. The first indication of the nuclear division is the disappearance of the membranous structure of the corbule, and the scattering of the chromatin masses into the ground substance of the nucleus. There the chromatin masses appear swollen and broken into pieces (Pl. X, fig. 6). The nuclear membrane becomes indistinct and the ground substance becomes denser in its constitution. At about this time, the division of the nipple takes place. The connecting strand continues to be elongated and the daughter nipples are separated by degrees. The nucleus and the strand, which were originally separated by some distance, are now gradually brought nearer to one another until they come to be united as is shown in Pl. X, figs. 7 and 8.

The strand is stretched into a slender filament, gradually attenuating towards the base of the nipple. The nucleus is found lying close by the filament, stretching along its whole length and fixed at both its ends. The chromatin masses become at this stage more or less long thread-like chromosomes, each consisting of several granules loosely arranged in a chain. The nucleus then assumes a spindle shape, lying closely by the filament, and the chromosomes also become arranged in a spindle along its middle portion (Pl. X, fig. 9). They then shorten and assume the shape of rods, provided with several slight constrictions, which exist throughout the rest of the division. The number of chromosomes counted varied from 10 to 14; but in the majority of cases they were found to be 12, which probably represents the actual number. Meanwhile the chromosomes are divided transversely at the equator and the ground substance is also divided at the corresponding place: the halves thus produced then move towards opposite poles along the filament, until they reach a position near the base of each daughter nipple (Figs. 10 and 11). The daughter nuclei are then separated from the filament and come to lie at a short distance behind the bases of the nipples, where the chromosomes break up into chromatin masses and the nucleus returns to its resting condition (Fig. 12). The filament becomes detached from the base of the nipple and soon disappears.

As the nipple is divided, the anterior part of the body becomes very much widened and flattened, so that the whole body assumes the shape of a turnip; the above-described processes taking place under the flattened surface of the anterior part. The flagella are not cast off, nor do they cease to move, and the organism usually continues to display a sluggish motion throughout the whole process of division.

Interesting and important questions are raised by the above-described phenomena of division, and concern the origin of the strand, and the rôle played by the daughter nipples. According to Foà (1904) and Kofoed and Swezy (1919), the strand is directly connected with the bases of the daughter nipples, and no structures such as centrosomes are recognizable at its ends. I have also failed to find any structure such as a centrosome and I am of the opinion that the rôle of division-centre is played by the daughter nipples. Further discussions of these points will be given in Part II.

As in the variety *japonica*, no forms suggesting the occurrence of any other modes of development have been observed in this variety. Forms closely resembling those taken by Porter to "present the best evidence of being the young" of *T. agilis* are met with in company with this form. But they are undoubtedly quite independent of *Trichonympha*, and I refer them to a new genus, *Microspironympha*.

II. *Pseudotrichonympha*.

Hartmann (1910) found three trichonymphids in a species of *Coptotermes*¹ in Brazil, and interpreted them as representing the young, the female, and the male, of a single new species which he called "*Trichonympha hertwigi*." Soon after the appearance of Hartmann's work, objections to his view were raised by Grassi (1911), and the "sexual forms" of Hartmann were made independent species, each being referred to a new genus, viz. *Holomastigotoides* and *Pseudotrichonympha*, proposed for the "female" and the "male" forms respectively. ("Male" and "female" contrariwise misprinted at that time, and corrected later.) In an article contributed to the *Handbuch der Naturwissenschaften* (Vol. III. 1913), Hartmann subsequently adopted the generic name *Holomastigotoides*, but did not give up his former conception as regards the relationship of the three forms. In *Coptotermes formosanus* three types are also found, and each of these resembles rather closely the corresponding one in the Brazilian termite. The results of my studies of these forms have convinced me that Hartmann's view is by no means right, and not only the "male" and the "female," but also the "young" forms, should be referred to independent genera.

Three more species of *Pseudotrichonympha* have been described recently. In his latest work (1917), Grassi described two new species of this genus, *P. magnipapillosa* and *P. parvipapillosa*, harboured by African (French Guinea) and Australian species of *Schedorhinotermes*. One more species was briefly described by Imms (1919)² from the Indian *Archotermopsis*. "*Trichonympha*" *leidy*, described by Kent (1885), probably belongs to this genus; but his account is not sufficiently detailed to make its precise determination possible. In spite of the resemblances, the species of *Pseudotrichonympha* in our termites shows some differences from the Brazilian, Australian, African, and Indian species, so that it should be regarded as new, and I have therefore proposed the name *Pseudotrichonympha grassii* for it. A discussion of Hartmann's view of the relations between the three types will be given later.

Pseudotrichonympha grassii sp. nov. (Plate XI, figs. 14–23).

This is one of the largest of the protozoa found in our termites, reaching a size visible by the naked eye. The body usually assumes a spindle shape, measuring 200–300 μ in length and 50–120 μ in breadth. Sometimes it assumes a slender lanceolate shape, and its length exceeds 500 μ in extreme cases. It is thus shorter and broader than Hartmann's species and those described by Grassi. It is markedly variable in shape as compared with *Trichonympha* and other forms found associated in *Coptotermes*.

In general organization it resembles *Trichonympha*, and the body may be distinguished into an anterior region or head, and a posterior region or body.

¹ Incorrectly called *Captotermes* by Hartmann.

² Incorrectly called "*Trichonympha* (*Holomastigotoides*)" by him.

The anterior region occupies only a small portion as compared with that of *Trichonympha*, and measures 30–40 μ in height, so that the posterior region is from six to nine times as long as the anterior. The anterior region may again be divided into a nipple and a bell, as in *Trichonympha*, though no circular fissure (“citartrosi” of Grassi) separating these parts is to be found in this species of *Pseudotriconympha*. The bell occupies some two-thirds of the anterior region, measuring some 20 μ in height and 40–50 μ in transverse diameter at its margin.

The nipple is of similar construction to that of *Trichonympha*, but differs in several details (Pl. XI, fig. 15). The axis of the nipple is not formed of a simple column, but consists of a tubule with a round ball situated at its anterior end. The layer surrounding the axis is thickly traversed by flagella and resembles the outer layer of *Trichonympha*, no layer corresponding to the inner layer being recognizable around the axis. The wall of the bell consists of two layers, just as in *Trichonympha*, the outer one being continuous with that of the nipple. The inner layer ends anteriorly at the edge of the base of the axis. It measures about 2 μ in thickness in the middle and becomes gradually thinner towards the ends. The tubular column is slightly thickened at both ends, the posterior end being the thicker. Its wall is fairly thin, and becomes also gradually thicker towards both ends, especially towards the posterior. The ball is solid, and its diameter is nearly equal to that of the anterior end of the tubular column. It fits closely into the opening of the latter; that is, only a hemisphere of the ball appears at the top of the axis, and the opening of the tubular column is perfectly closed. Both the wall of the tubule and its contents are transparent, homogeneous, and structureless, no granules as seen in *Trichonympha* being recognizable in the contents of the axis of this organism. The wall and the contents, moreover, are of nearly the same refractive index, so that these two constituents of the axial column are not easily distinguishable. The ball is usually indistinctly recognizable in fresh specimens, perhaps owing to its having nearly the same refractive index as the contents of the cap.

The layer around the axis is of the same structure as the outer layer of *Trichonympha*, but its ground substance is not of uniform density in this organism. It is dense immediately around the column and has the same appearance as the outer layer of *Trichonympha*, in both fresh and stained preparations. The peripheral half is, however, much thinner. The boundary between these two layers is more or less distinctly discernible in some individuals. The flagella are shortest at the anterior and longest at the posterior region of the head, the free portions of them measuring 6–8 μ and 25–30 μ in these two regions respectively. They are more markedly inclined backwards than in *Trichonympha*, and the cap is much taller, its edge being found near the middle of the nipple. In living specimens the cap can be seen to undergo changes of shape in this species, but it is rather rare to see it assuming the shape of a hemispherical bowl.

In fixed preparations the ball is very feebly stained by iron-haematoxylin, but the wall of the tubular column is stained deeply, while its contents stain very feebly or not at all. The outer surface of the tubular column is stained intensely, and a granular appearance is here recognizable in some specimens. The boundary of the outer and the inner layers in the bell is also stained deeply, and shows a granular appearance. The inner layer appears quite the same as the corresponding layer in *Trichonympha*, in fresh specimens as well as in stained preparations. It cannot be doubted that the flagella of the nipple arise from the above-mentioned deeply stainable zone lying immediately on the surface of the axis, and those of the bell from the similar zone at the base of the outer layer.

It may be added here that the descriptions of the anterior region in the species studied by Grassi and Hartmann do not agree with mine. According to Hartmann, in the Brazilian species, the column and the body at the top are both solid, and the latter is not spherical but hemispherical, lying on the flat surface of the anterior end of the column. In the African and the Australian species, Grassi describes both structures as hollow, filled with liquid. As regards the layers surrounding the axial core in the nipple and those in the wall of the bell, moreover, the descriptions of these species do not agree with mine. According to Hartmann, there is no layer corresponding to the inner layer, both in the nipple and in the bell. That is to say, the axis of the nipple of "*Trichonympha hertwigi*" is directly surrounded by a layer corresponding to the outer layer of *Trichonympha*, just as in our species, but the wall of the bell consists also of a single layer, thus differing from our species. Grassi, on the other hand, distinguishes two layers, both in the nipple and in the bell, and he believes that the flagella arise at the base of the inner layer. I am not quite certain whether or not such differences really exist between these species, but I doubt the accuracy of the authors' observations as regards some of the above details.

The entire posterior region of the body is covered by fine flagella densely distributed, excepting only over a small portion at the posterior extremity. They are of almost uniform length over the whole surface, measuring 16–20 μ . They are arranged in numerous rows, giving the body an appearance as if it were beautifully striped. Forming the body wall there is one distinct layer, measuring about 2 μ in thickness. It exists from the edge of the bell to the posterior end, but becomes extremely thin over the small bare area at the hinder extremity. The layer is of the same structure as the basal part of the outer layer of the bell, and at the edge of the bell it can be clearly made out that the two are continuous. The basal granules of the flagella are localized at the base of the layer, where a distinct zone of other minute granules, deeply stainable with iron-haematoxylin, is recognizable. The basal portions of the flagella, being embedded in rows in this layer, appear to form a kind of band or plate. The rows of flagella are arranged spirally, uniformly separated by some 2 μ in the middle region, but becoming gradually closer towards the

margin of the bell and the posterior end. The winding of the spiral rows is left-handed (laetotropic)¹. The outer surface of the body wall seems to be destitute of any specially differentiated membrane.

The endoplasm appears minutely granular and no alveolar structure is recognizable. Beneath the body wall, a zone of coarse and deeply stainable granules, as seen in *Trichonympha*, is recognizable. A thin but distinct zone of clear and structureless substance between the basal layer of the body wall and this layer of granules is also present in both the anterior and posterior regions.

The organism seems to be voracious, and contains a greater or less abundance of food débris, consisting chiefly of wood fragments. The food bodies are commonly limited to the portion posterior to the nucleus, but they may be found very rarely in the region between the nucleus and the anterior end.

The nucleus is found usually a little anterior to the middle of the body, though sometimes situated far posteriorly, and there is no structure, such as a corbule, connecting it with the body wall. It is round in shape, measuring 16–20 μ in diameter. Its wall is peculiar in this form, appearing very thick and abnormally tough. In fresh specimens the wall is markedly refractive, and the internal structure is not visible. In the majority of cases the contour is not even, and a wide groove or distinct folds may be commonly found running over the surface (Pl. XI, fig. 14). This peculiarity of the nuclear wall can be seen clearly in living specimens. The inner structure of the nucleus differs somewhat from that described by Hartmann and Grassi. It consists of a network of chromatic threads and clear ground substance. In some individuals the network is loose, while in others it is rather dense, presenting a spongy appearance. It is a peculiarity of this species that the network forms a more or less smooth and distinctly contoured mass, leaving a space under the nuclear wall (Pl. XI, figs. 14 and 17).

Dividing forms are frequently met with in this species. The process of nuclear division is peculiar, and different from that of the preceding genus. At the first stage in division, a peculiar structure makes its appearance at the anterior region, as is shown in Pl. XI, fig. 18. It is a thread hanging in the endoplasm, bent at several points, and provided with a small sphere at its free end. The opposite end of the thread could not be made out, because of the feebly stainable character of the thread and owing to the dense zone of granules distributed under the wall of the bell. However, it cannot be doubted from its behaviour that it is connected with the base of the axis of the nipple. The sphere and the thread stain more or less faintly with iron-haematoxylin. The sphere probably approaches the nucleus rapidly, until it comes to lie on its surface, and the thread then appears as a straight or slightly wavy line.

¹ The direction of the spiral rows of flagella, in all of the other accompanying forms found in the intestine of *Coptotermes formosanus*, is just the reverse of that in this form, namely right-handed (dextrotropic). In Hartmann's figure of his "male" form the rows are drawn as left-handed spirals, while those of his "female" forms (*Holomastigotoides*) are shown partly as left-handed and partly as right-handed.

Division takes place when the sphere and the nucleus are brought together, the sphere, the thread and the nipple almost simultaneously dividing into two. The axial column of the nipple splits longitudinally into two halves, the split beginning at the posterior end and extending forwards. The surrounding layer also divides, each half being accompanied by one of the daughter axes. The ball at the top of the column is not divided completely at this stage, but is constricted first into a dumb-bell, and remains connecting the top of the daughter axes until just prior to the completion of the whole process. When the little sphere at the end of the thread divides, the two daughter spheres are situated on the surface of the nucleus, each connected with the base of the daughter nipple by means of a daughter thread, formed apparently by a splitting of the original single thread. The most remarkable feature observable at this stage is a thick and distinct strand connecting the daughter spheres, on the surface of the nucleus (Pl. XI, fig. 19). It is identical in its structure with the strand seen in *Trichonympha*, though it is, in *Pseudotrichonympha*, more conspicuous and distinctly fibrous. The above processes must take place very quickly, since I have not yet seen a specimen with the nipple already divided but the strand not yet found on the nucleus. Owing to its fineness and its faint stainability, the thread may frequently be invisible, but the strand never escapes the observer's eye. In this respect, Hartmann's description appears somewhat singular to me. He described some individuals with divided nipples, but in none of these forms does he seem to have noticed either the strand or the thread connecting the nucleus with the nipple. It is certain, however, that only a few forms in an early stage of division were observed by Hartmann, and he did not follow the subsequent processes. Thus it is not clear whether a strand makes its appearance in the Brazilian species later than in our species, or whether it does not appear at all.

The strand is now gradually elongated. The nuclear membrane persists throughout the whole division process, and the spheres are always attached firmly to it. Consequently elongation of the strand is accompanied by an alteration in the shape of the nucleus. The strand lies transversely and the nucleus is disposed, in the majority of cases, along the whole of its length, and appears as a segment of a circle (Pl. XI, fig. 20). Hand in hand with the alteration of the shape of the nucleus, changes occur also in the chromatic network. Chromosomes are formed, by the chromatic substance forming an irregular network or a spongy mass, and coming gradually to assume the form of more or less distinct threads with nodular thickenings. These chromatic threads become gradually distinct and uniform in thickness, coiled in the nucleus, appearing like the spireme in metazoan cells. Finally they resolve themselves into a large number of chromosomes, arranged along the strand and fixed to the spheres at both ends. They then split transversely into pairs of daughter chromosomes. I think it probable that the chromosomes do not split simultaneously, but are divided one by one. They are of nearly uniform thickness, but their surface is rough. The daughter chromosomes fixed to the polar

spheres differ remarkably from each other in their length; and they are very difficult to count, so that I cannot give a definite figure for their normal number. The difficulty is chiefly due to the fact that some of the chromosomes, where they are fixed to the sphere, are bent at one or more points; and moreover, some of them are coiled around each other. The number seems to be more than 12.

While the above processes are going on, both poles of the nucleus become gradually thickened and rounded. At this stage the chromosome-formation is completed, and a constriction appears at the middle of the nucleus, indicating the plane of division (Pl. XI, fig. 23). The strand becomes still more elongated until it reaches twice or thrice the length of the diameter of the resting nucleus. The just divided daughter nuclei are then found attached to its two ends (Pl. XI, fig. 21). Finally the strand is broken and the nuclei are detached from it, leaving the spheres behind, hanging from the nipples by means of their threads, with the broken remains of the strand still attached. The mode of breaking of the strand is noteworthy. The elongated strand is usually seen to be conspicuously swollen in the middle, and it is on either side of this swollen portion that the break occurs. Thus the strand is broken into three, and its fibrous structure is beautifully demonstrated at the fractured places. It is at this stage that the constricted ball on the axial column of the nipple is severed, and the division of the nipple is thus completed. The division of the nipple is quickly followed by the division of the bell. Complete division of the body seems to be effected rather slowly, and individuals with a divided anterior portion, such as that shown in Pl. XI, fig. 22, are often observed in fresh preparations, remaining undivided for some time. In the young animalcules soon after division, remnants of the strand and the thread may be seen. Unfortunately I could not ascertain the fate of these structures. Reorganization of the normal structure in the young seems to be carried on quickly.

III. *Teratonympha*.

An organism with a remarkable structure is found in the *Leucotermes* of both Japan proper and Formosa, and for it I propose the new generic name *Teratonympha*¹. It shows so many peculiarities in its organization that it seems at first sight to differ greatly from all the hitherto described protozoa of termites. However, when the organization of the head is carefully studied, a fairly close relationship can be clearly made out between this form and the preceding genera; and the arrangement of its flagella can be homologized with that seen in *Holomastigotidae*.

The individuals in *Leucotermes speratus* of Japan proper are somewhat different from those in *L. flaviceps* of Formosa, and the rather slight but distinct differences between them can be recognized constantly; so I have

¹ In my original paper in Japanese (1917) I wrote this name as "*Teranympha*"—an orthographic error which I now correct.

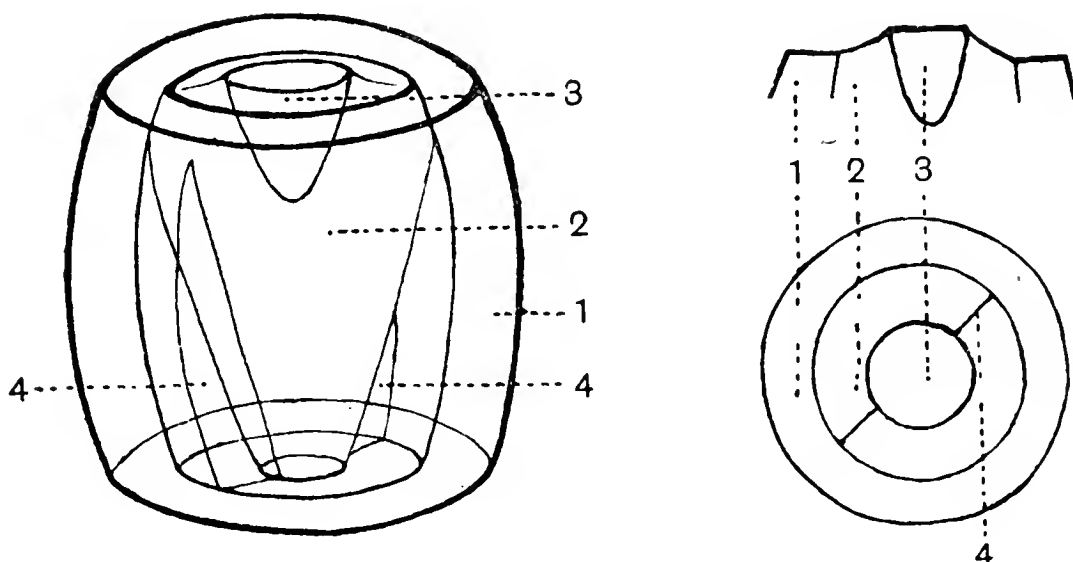
distinguished them as *Teratonympha mirabilis* (type species) and its variety *formosana*, respectively.

- (1) *Teratonympha mirabilis* gen. nov. et sp. nov. (Plate XII, figs. 26–31, and Text-figs. A and B).

This is one of the largest of the protozoa in Japanese termites and much the largest of the forms found in *Leucotermes*; and its large size, coupled with its monstrous appearance, at once attracts the observer's attention. Ordinarily the organism assumes the shape of a club, being rounded at the anterior end and somewhat pointed posteriorly. The differentiation of the parts of the body of this organism is far more remarkable than in *Trichonympha* and *Pseudotriconympha*. The anterior portion of the body has a remarkably complicated construction, and the posterior region appears very peculiar, being apparently segmented like a cestode. The specially differentiated anterior part, or head, has the shape of a concavo-convex lens. The body is widest near the head and gradually decreases in diameter towards the posterior end. The organism usually measures 200–250 μ , but sometimes 300 μ or more, in length, and 40–50 μ in width at the widest portion. In the posterior region the body wall is not smooth, but is provided with numerous transverse and more or less prominent and sharp ridges, which are inclined backwards. The regular metameric arrangement of these ridges gives the body the segmented appearance which suggests the proglottids of a tape-worm. The appearance of the endoplasm also tempts one to think that the body is made up of successive segments, as in the cestodes. But careful observations reveal that the differentiation is limited to the body wall, and no septa are to be found crossing the endoplasm. The number of the ridges varies from 18 to 30, or sometimes even more, in proportion to the length of the body. As the animalcules are subjected to unfavourable conditions, their shape changes more or less quickly to that of a spindle or turnip, until finally they become rounded and burst. The flagella are very conspicuous in this form. Very long ones are found closely distributed at the head, as in the preceding genera. On the surface of the body, however, they are not distributed diffusely, but are arranged in parallel rows, each of the transverse ridges being provided with one row of them.

The head consists of two parts; namely, a thick axial column or cylinder and a peripheral layer traversed by numerous flagella, arising at the surface of the column. The peripheral layer encircles the axial column from top to base, leaving the frontal surface uncovered, and becomes gradually thinner towards the sides as it curves backwards. The axial column itself is ordinarily barrel-shaped, being slightly narrowed towards the top and the base. It measures some 10 μ in breadth and 15 μ in height. Its shape is, however, variable to a certain extent, and it is often narrowed at the anterior end only. Its complicated organization is shown in Text-fig. A. As it is quite transparent, the details of its structure can be made out in the living organism.

Differential staining of the several parts is not easy, and stained preparations are not so suitable for studying the structure of the head. The principal parts of the axial column are a circumferential wall or rind of a tubular form, and a central cone, or core, fixed within it. The tubular rind is of a nearly uniform thickness, measuring $1.5-2\mu$; and circular openings, measuring $4-5\mu$ in diameter, are found at both its ends. The conical core is widest at the anterior end, fitting into the upper opening of the tubular rind and gradually decreasing in thickness posteriorly so that it resembles an inverted cone, having the greater part of its surface separated from the tubular rind. The conical core itself is not composed of a single substance, but a body assuming the shape of an inverted cone or hemi-ellipsoid is found embedded in its anterior or basal portion. The main portion of the core completely covers this smaller conical body, leaving only the flat surface of the latter, corresponding to the basal plane of the cone, uncovered. The front end, or base, of the smaller conical body, and the tubular rind together form a smooth anterior



Text-fig. A. Diagrammatic representation of the axial core at the anterior end of the body of *Teratonympha*. 1, tubular rind; 2, outer cone; 3, inner cone; 4, vertical partition.

surface for the whole structure; but the central cone is a little more advanced in position than its surrounding tube, so that the frontal surface of the main part of the axis slopes gradually from the junction of the two cones towards the boundary between the external cone and the tubular rind. The interval between the core and the rind is more or less spacious, and is filled by a structureless protoplasmic substance of dense consistency. Though the rind and the greater portion of the conical core seem completely separated from each other, careful observations show that there is a distinct vertical partition on both sides, stretching from the posterior end of the whole axis to the point where the tubular rind and the conical core are in contact anteriorly. The two partitions are so situated as to lie in one plane, or nearly so, and divide the space between the apex of the cone and the tube into two nearly equal halves.

The tubular rind takes iron-haematoxylin very feebly or not at all, excepting a zone of limited breadth at the inner surface, which is stained rather

more deeply, just as in the inner layer of the nipple and the bell of *Trichonympha*. The outer surface takes dyes intensely, and shows an appearance of an aggregation of minute and very deeply stained granules, indicating that the flagella arise therefrom. Thus it seems probable that the rind is the homologue of the inner layer of the head of *Trichonympha* and *Pseudotriconympha*. The conical core and the contents of the space around it stain more or less deeply. The outer surface of the cone, and the vertical partition connecting it with the inner wall of the tubular rind also stain deeply, but no granular appearance is there visible. Differential staining of the inner and outer cones of the core at their apices is not easy, but the outer seems to stain a little more faintly.

The peripheral part of the head is identical in structure with the outer layer in the anterior region of *Trichonympha*. It is closely traversed by flagella, which arise at the junction with the central axis. Owing to the flattening of the head, the proximal ends of the flagella embedded in the peripheral layer are very long, so that their distal or free ends are nearly equal to the embedded portions in length. The flagella are shortest anteriorly, and gradually increase in length posteriorly: the longest ones measure 20–25 μ , including the embedded portion. Behind the head, a thin but distinct layer is noticed lining its concave surface. The flagella do not arise from this layer, but run parallel to it.

The anterior surface of the whole axis is apparently naked, and provided with no special structure, such as the cap on the nipple in the preceding genera. Careful observation, however, shows that there is a thin membrane lying on the frontal surface of the head—this membrane being hardly noticeable, owing to the absence of any space between it and the frontal surface.

In vigorous animalcules the head is either swung actively from side to side, or is held straight and the axial column alone displaced backwards and forwards along the long axis of the body. In the former case, the shape of the head becomes markedly asymmetrical; in the latter, the central portion of the head is alternately drawn in and pushed out in rapid succession.

From the above descriptions, it will be recognized that the head of this form is homologous in organization with the anterior region of *Trichonympha* and *Pseudotriconympha*. As already indicated, the peripheral part traversed by the flagella, and the tubular rind of the axial column, are respectively comparable with the outer and the inner layers of the anterior part of *Trichonympha*. It seems probable also that the conical core and the inner conical body, at its anterior or basal end, are homologous respectively with the columnar axis, and the ball at its anterior end, in the nipple of *Pseudotriconympha*. As regards the contents of the space between the outer cone and the tubular rind, comparative studies of allied forms afford strong evidence that it is homologous with the contents of the axis of the nipple in *Trichonympha*. A discussion of the nature of these structures will be given in detail in Part II.

A single large nucleus is situated at a short distance behind the axial column. It appears usually oval in shape, measuring 10μ or rather more in diameter. It is not suspended freely in the endoplasm, but is enveloped in a sac, the nuclear sac, hanging from the base of the axial column of the head. The sac has the shape of a flask with an ovoidal or globular body and a slender neck. It is, moreover, provided with a flange-like extension in the horizontal plane at the equator of its globular portion. The nucleus almost fills the main body of the sac, which fits itself very closely round the nuclear membrane; and consequently the sac is usually inconspicuous on the surface of the nucleus, which seems to be united to the base of the axial column by means of a membranous tube at its anterior pole, and provided with a horizontal extension at its equator. This horizontal flange or plate is distinct in the vicinity of the nucleus and becomes gradually indistinct towards the body wall, so that its entire course can be clearly followed only in good preparations. The tubular neck of the sac varies somewhat in length, according to the condition of the head and the size of the nucleus. During the movements of the animalcule, the shape of the nucleus is also frequently modified by the pressure exerted by the sac, especially when the nucleus itself is large. The nuclear sac is continuous at its anterior end with the substance filling the space between the rind and the core of the axial column of the head. I am of the opinion that the sac is homologous with the corbule of *Trichonympha*, which keeps the nucleus in connexion with the body wall.

The nucleus is of the same type as in *Trichonympha*. No trace of linin reticulum is to be seen, and fairly large chromatic masses are found assembled at the middle portion, leaving a wide zone of clear ground substance peripherally. In some forms the chromatic bodies are distinct in outline, as is common in *Trichonympha*; while in some others they are united to form a rough network, as in *Pseudotriconympha*. A single nucleolus is clearly discernible in nearly all individuals, situated near the centre of the nucleus and surrounded by a clear space.

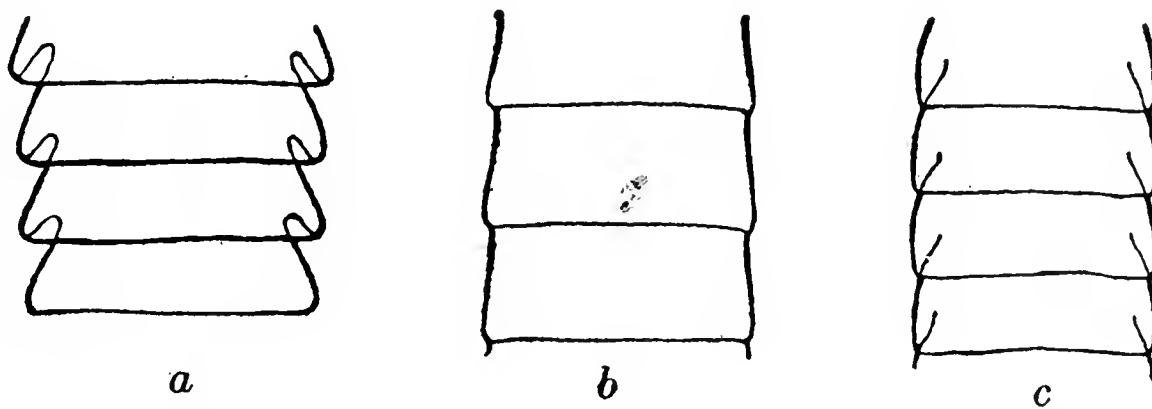
As already noted, the body is provided with numerous transverse ridges on its wall. In the normal state each of the ridges has the shape of a drawn out and flattened fold, inclined backwards so that a distinct groove is formed under it (Text-fig. B, *a*). The ridges, however, are not necessarily distinct in every individual, and when the body is abnormally elongated they almost disappear (Text-fig. B, *b*). In some cases, on the other hand, they are pressed so closely against the body that the grooves entirely disappear, and the surface then appears almost smooth (Text-fig. B, *c*).

In the endoplasm two portions are distinguishable. The main portion is similar to that of *Trichonympha* or *Pseudotriconympha*, being more or less coarsely granular and appearing grey in colour in the living animal. The ridges, on the other hand, consist of a transparent and structureless protoplasm of dense consistency.

The body wall differs from that of the preceding forms, and the mode of

arrangement of the flagella is also peculiar. There is no special layer, as seen in the preceding forms, on the body wall, but it is provided with a thin but rigid membrane or periplast. The flagella are not distributed diffusely, but are arranged in distinct transverse rows, and become free at the edge of each of the transverse folds. A band of deeply stainable granules is distinctly discernible at the bottom of each groove formed between two successive ridges. It is from this band that the flagella arise, and it can hardly be doubted that a row of basal granules exists somewhere in the band, though its precise location is not clearly visible. The flagella of this form differ from ordinary flagella or cilia in their relation to the body wall, their basal portions being adherent to the periplast. They run backwards, fixed on the surface, until they reach the summit of the fold, where they become free. Each flagellum measures about 30μ in length, the free portion constituting about two-thirds of the entire length (Pl. XIII, fig. 53 a).

In the anterior part of the body, some peculiar filamentous structures, varying in size as well as in number, are usually seen in the endoplasm. They



Text-fig. B. Diagrammatic drawing of various conditions of the ridges on the body wall of *Teratonympha*. Explanation in the text.

are commonly bent at the anterior end, the main portion of the filament usually lying longitudinally. They are composed of a transparent substance showing sometimes a fibrous structure, and are covered thickly by minute but deeply stainable granules. From two to five may be present, and they are invariably found close behind the nucleus (cf. Pl. XII, fig. 24).

Dividing forms are occasionally met with in this species as well as in the Formosan variety. The process of division is analogous in essential points to that of *Trichonympha* and *Pseudotriconympha*, but is more complicated. In individuals beginning to divide, the body is shortened and widened, especially at the anterior end, so that the organisms become turnip-shaped. The axial column of the head is divided longitudinally. Pl. XII, fig. 28 represents an individual in an early stage of the process: the axial column is already split into two equal halves, and a filamentous strand, identical with that seen in *Trichonympha*, is seen lying near its base. The two ends of the strand of this form are separated from the column; but there is a thread-like structure connecting each end of the strand with the base of each daughter column (Pl. XII, fig. 29), like that seen in *Pseudotriconympha*. At either end the strand is provided with

a minute sphere of dense protoplasm containing a deeply stained granule. The formation of this strand seems to occur very quickly, for specimens at this stage are extremely rare. In Pl. XII, fig. 27 is depicted an individual in an earlier stage of division: there is a horseshoe-shaped structure stained moderately by iron-haematoxylin, with a globule, stained deeply, at each end, and a thread connecting it with the base of the recently divided axial column. The horseshoe-shaped body is probably an early stage in the development of the straight strand.

As in *Trichonympha*, indications of division are recognizable in the nucleus earlier than in the head. The first sign of division seen in the nucleus is the disappearance of the nuclear sac. In the nucleus, now free in the endoplasm, the chromatic bodies are resolved into fragments, the ground substance becomes gradually denser, and the nuclear membrane becomes indistinguishable (Pl. XII, fig. 28). While the above processes are going on in the nucleus, the axial column is divided, and the strand makes its appearance and continues to grow thicker and longer. The nucleus now gradually draws near the strand, coming to lie closely under it, and finally becomes united to it, as in *Trichonympha*.

At this stage chromosome formation is completed. The chromosomes are arranged rather regularly side by side. The mode of chromosome formation seems analogous to that of *Trichonympha*. The chromatic granules are so arranged as to form a certain number of chain-like threads. In some individuals several pairs of chromatic threads are observed lying closely parallel to each other (Pl. XII, fig. 27), and I am inclined to take this appearance to indicate that the daughter chromosomes are formed by longitudinal splitting. The fact that the chromosomes when arranged in a single plane during later stages, such as that shown in Pl. XII, fig. 29, are equal in length to those in the daughter nuclei, seems to support the above supposition, and show us that the splitting of the chromosomes occurs early in this species. In this species, the number of chromosomes counted varied from 20 to 30. I have not been able to determine their number exactly. They are not uniform in thickness but nodular, and do not differ distinctly from each other in their length.

As the strand and the nucleus are brought into connexion, the daughter chromosomes move towards the opposite poles, and the division of the ground substance then follows. The fate of the strand in this organism is different from that of the similar structure observed in *Pseudotriconympha*. It does not remain long attached to the daughter nuclei, but becomes detached from them, and does not break into three as in *Pseudotriconympha*. In Pl. XII, fig. 30 an individual (only two-thirds of the body depicted) in a late stage of division is shown. The strand is here seen detached from the nuclei, and one of the latter is seen still connected with the daughter column by means of its filament. In each daughter nucleus, now just separated, we see a large spherical mass of dense protoplasm, with a granule near one of its poles: the chromosomes are radially arranged, fixed to the spherical body at the side opposite

to that where the granule is situated, and embedded in the ground substance, which now has a distinctly oval shape (Pl. XII, fig. 30). The thread connecting the daughter nucleus with the daughter nipple soon disappears, and reorganization of the nuclear structure and the reformation of the nuclear sac are carried on. In Pl. XII, fig. 31, is shown a young nucleus undergoing reorganization. Here we see the ground substance of the nucleus becoming reniform, with the chromatic threads embedded in it, and a distinct clear zone surrounding the whole mass. The latter structure is peculiar to this form. The boundary between the clear zone and the endoplasm is distinct, indicating clearly the existence of a membrane, and one deeply stainable granule is found on its anterior border. As regards the nature of the above-mentioned structures, I believe that the reniform mass represents the nucleus in process of reorganization, and the membrane at the outer boundary represents the new nuclear sac. Unfortunately I could not completely trace the formation of the new nuclear sac; but I believe that the neck and flange-like part are developed from the above-mentioned membranous wall, and hand in hand with the process of reorganization, the clear zone under the membrane becomes reduced, until it finally almost disappears around the nucleus. Some further discussions of these points will be given in Part II.

(2) *Teratonympha mirabilis* var. *formosana* var. nov.
(Plate XII, figs. 24 and 25).

As already remarked, the form of this genus found in the termites of Formosa differs from that just described, occurring in Japan proper, and was regarded as a distinct variety. The variety *formosana* is distinguishable from the type species by the following characters (see Pl. XII, fig. 25). The neck of the nuclear sac is shorter, being usually half as long. Further, the main body of the sac enveloping the nucleus is found in the preceding form applied closely to the nuclear membrane, so that the sac is not usually clearly distinguishable from the nuclear wall; but in this variety it is separated from the nuclear membrane, leaving a distinct space between them. Both these differences are clearly visible in the living animals, as well as in stained preparations.

Dividing forms are rather frequently met with in this variety, and the process of division is identical with that observed in the preceding form.

IV. *Microspironympha*.

This is a new genus proposed for a small form harboured by the Formosan species of *Leucotermes*. Some of the forms taken by Leidy (1881) and Porter (1897) to represent possible young stages of *Trichonympha agilis* resemble this form closely, but owing to the insufficiency of the descriptions of the American authors, the identification of their forms is hardly possible. As compared with *Trichonympha*, our form is remarkably simple in organization, and differs so distinctly that the existence of a genetic connexion is hardly

imaginable. Their independence cannot be doubted also from the fact that the present form is found accompanied by *Trichonympha* in the Formosan termites only: in the termites of Japan proper, *Trichonympha* does not accompany any form of this type. It is possible that some of the forms described by Hartmann (1910) as young stages of *Trichonympha* also belong to this genus, or to *Spirotrichonympha* of Grassi; but from Hartmann's description and figures of the anterior end of his organisms, it is impossible to identify them with certainty. In my former paper (1917) in Japanese, I referred this organism to the new genus *Spiironympha*. Grassi (1911), however, had previously proposed *Spirotrichonympha* for a different form; and therefore, as my name was merely an abbreviation of the same name, in order to avoid confusion I now propose to call the present form *Microspironympha*.

Microspironympha porteri gen. nov. et spec. nov. (Plate XIII, figs. 32–38).

This form is somewhat variable in shape, but commonly fusiform, the length being about twice the breadth. The anterior half of the body is provided with a fairly rigid periplast, and displays little alteration of shape, being usually in the form of a tall cone. The posterior half, on the other hand, is variable in shape. It is rounded in some forms (Pl. XIII, fig. 33), while in others it is prolonged and pointed at the end, giving a spindle-like appearance to the body. In some forms, the posterior end is drawn out into a slender tail-like appendage, variable both in shape and in size. When carefully studied, a slender rod, resembling the axostyle of some species of trichomonad flagellates, is recognizable running the entire length of the tail-like portion (Pl. XIII, fig. 34). The rod is transparent, slightly refractive, and not stained with any dyes. It is only in the slender portion that it is clearly recognizable, and it is never visible in individuals with rounded posterior ends. It is probable, however, that it exists in the majority of individuals, but is invisible in plump forms, being hidden in the endoplasm. Though the anterior end of the rod cannot be made out in any individuals, it may be safely concluded from its behaviour that it is of limited length and is not connected firmly with any part of the body at its anterior end. In this respect it is less developed than the axostyle of trichomonads, and neither serves as a skeleton for the whole body, nor for the control of its movements as in these flagellates.

The organism is small in size for a trichonymphid, spindle-shaped individuals measuring 20–55 μ in length and 10–30 μ in breadth, piriform ones 20–40 μ in length and 18–30 μ in breadth (the tail-like portion being excluded).

Under the periplast of the anterior portion, several narrow bands, consisting of minute granules deeply stainable with iron-haematoxylin, are noticed running in parallel spiral lines. The number of these narrow bands appearing in side view varies commonly from 6 to 8. Careful study shows that there are really four of these bands starting at the anterior tip of the body and turning round spirally towards the posterior portion (Pl. XIII, fig. 35). The direction of the spiral of the bands is always dextrotropic, as in the forms

described by Porter. The bands do not reach the posterior end, the hinder portion of the body being free from them. In some individuals, measuring some 20μ in length, the anterior region provided with the bands is nearly equal to or rather larger than the posterior region which is destitute of them. As the animalcule grows larger, the bandless posterior portion is chiefly increased in size, so that in large individuals the anterior region is commonly but little larger than in small forms, while the posterior region is relatively very voluminous, its length measuring two, three, or more times that of the anterior region.

It is from these spiral bands that the flagella arise, the basal granules of the flagella being situated in them. The flagella are rather conspicuous in this organism. They are shortest anteriorly and increase in length towards the posterior, the hindmost being some three times as long as the shortest at the anterior end. In large forms, measuring $30\text{--}40\mu$ in length, the shortest flagella measure some 10μ and the longest ones some 30μ , so that a large number of them pass over the hinder part of the body. In small forms the flagella are proportionally somewhat longer. The shortest flagella situated anteriorly are directed forwards and are swung vigorously. The longest ones, on the other hand, are directed backwards: they lie more or less closely on the surface of the body, and do not move so actively.

The endoplasm seems less granular than in most other trichonymphids. It is remarkably viscid and a large amount of food débris is commonly found in the posterior region. The most remarkable character of this form is that it seems omnivorous, and not only wood fibres but other protozoa are found in the endoplasm, probably ingested as food. It is very common to see individuals with their endoplasm gorged with organisms such as spirochaetes, small trichonymphids, or other flagellates, the shape of the body being sometimes remarkably altered owing to the large size of the ingested organisms.

The nucleus is situated near the anterior end. It is oval or round, measuring $4\text{--}5\mu$, or sometimes as much as 7μ in diameter. It contains usually a pair of chromatic bodies, hanging from the anterior surface and suspended in the homogeneous ground substance (Pl. XIII, figs. 32–34). In the majority of cases, these chromatic bodies are club-shaped, and are independent of one another, hanging side by side. In some forms, however, their posterior ends or the greater part of them are fused together, so as to form, in extreme cases, a body of an oval shape, attached to the anterior wall of the nucleus by means of two mammillary projections. The chromatic bodies usually stain very deeply and almost homogeneously, though in good preparations a more faintly stained ground substance with intensely stained granules embedded in it can be distinguished. The nucleus is surrounded by a dense and structureless substance and is very distinct in its contour, though the membrane is not clearly recognizable.

The nucleus is not suspended freely in the endoplasm, but is connected with the anterior tip of the body by means of a special structure. This is a

distinct tubular organ of uniform thickness, measuring some 1μ in breadth and $4-7\mu$ in length. It is clearly visible in living specimens as a transparent and refractive rod. It takes iron-haematoxylin intensely, its wall appearing as though consisting of closely packed granules. In the majority of cases, its posterior end is directly connected with the nuclear wall, and it is at their point of contact that the chromatic bodies are attached. In many cases distinct granules can be recognized at the two points of connexion of the chromatic bodies and the nuclear wall. The tubular structure is neither closed nor provided with a special structure at the anterior end; and it is from the edge of its circular anterior opening that the characteristic bands of deeply stainable granules lying under the periplast originate (Pl. XIII, fig. 35). The substance forming the contents of the tubule is dense and homogeneous, and takes eosin somewhat intensely. As described above, the nucleus is surrounded by a layer of dense protoplasm, and this layer is especially conspicuous in the front part of the nucleus; that is, round the posterior part of the tubule. In large-sized forms the tubule is frequently detached from the nucleus, or its hinder portion is indistinct, and it is apparently kept in connexion with the nucleus by means of the particularly dense surrounding mass of protoplasm (Pl. XIII, figs. 36 and 37).

Dividing forms are met with very rarely in this species, and despite my eager search, only a few forms in later stages of the process were observed. In Pl. XIII, fig. 38, is shown an individual in a stage just before the completion of division. There we see a filamentous structure, its central portion forming a distinct filament, and its ends consisting of deeply stained granules arranged in a chain. Two distinct chromosomes are found symmetrically situated near the ends of the filamentous portion, embedded in a mass of homogeneous protoplasm. Unfortunately I have been unable to find sufficient material to ascertain the origin of these structures. It cannot be doubted, however, that the process of division is analogous in essential points to that of *Trichonympha*. Further discussion of these points will be given in Part II.

V. *Holomastigotoides*.

This is the genus proposed by Grassi (1911) for an organism found in the Brazilian species of *Coptotermes*, and formerly distinguished by Hartmann (1910) as the "female" or "Form B" of "*Trichonympha hertwigi*." As already remarked, there are sufficient reasons to disprove Hartmann's conception regarding the genetic relationship between his "male" and "female" forms, and Grassi's action in establishing the genus was undoubtedly right. As regards the affinity of his "female form" with other forms, Hartmann remarked: "sollte sie sich bei weiteren Untersuchungen wider Erwarten doch als eine besondere Art herausstellen, so müsste sie wohl in der Gattung *Dinenympha* gestellt werden." *Dinenympha*, however, represents a totally different type, provided with four or eight peculiar flagellar cords and differing also in other important characters.

As noted already, forms presenting a type of organization closely resembling that of Hartmann's "female form" are also found in *Coptotermes formosanus*, in company with the forms representing the type of his "male form," or *Pseudotrichonympha*.

Three species of *Holomastigotoides* are distinguished by Grassi (1917)—*H. hertwigi* Hartmann, *H. mirabile* Grassi (harboured by *Coptotermes sjöstedti* of French Guinea), and *H. hemigymnum* Grassi (harboured by *Coptotermes lacteus* of Australia). The chief differences between them seem to be in the arrangement of the spiral bands of flagella. According to Grassi (1917), *H. hertwigi* has very numerous bands, *H. mirabile* has only 12, while *H. hemigymnum* often has no bands over a large part of the posterior region of the body. Grassi's estimate of the number of bands in *H. hertwigi* is based upon Hartmann's Figure 44, which shows 20; but Hartmann's description and figures are not sufficiently accurate to warrant any definite conclusion regarding their number. Another of his figures (Fig. 46) is said by Hartmann himself to show 14 bands, but the drawing is not at all clear. The species of *Holomastigotoides* found in *Coptotermes formosanus* is similar in size to that described from the *Coptotermes* of Brazil by Hartmann; but owing to the incomplete and inadequate description which he has published, it is impossible for me to determine with certainty whether his species and mine are the same or different. From Grassi's brief descriptions it seems certain that his two species differ from mine, and I propose therefore to name my species *H. hartmanni*, provisionally regarding it as new.

Holomastigotoides hartmanni sp. nov. (Pl. XIII, figs. 43–52).

This is a large and plump form, commonly oval or elliptical in outline, and slightly narrowed at the anterior end. It usually measures 50–140 μ in length, but sometimes as much as 170 μ ; its breadth being usually 30–80 μ , exceptionally attaining 100 μ . On the greater portion of the body a distinct and well-developed periplast is recognizable. The shape of the body is not changeable. (See Pl. XIII, fig. 43.)

Close spiral bands of deeply stainable minute granules, as seen in *Pseudotrichonympha* and *Microspironympha*, are found over the greater portion of the body, excepting a small area at the posterior end, equal to about one-ninth of the whole body. The bands contain the basal granules of the flagella, as in the other genera. The number of bands appearing on one side of the body varies from 30 to 50, according to the size of the organism. In the Brazilian species they seem less numerous and distributed more diffusely than in our forms, and in *Holomastigotoides hemigymnum* described by Grassi (1917) a large portion of the body remains free from bands, as mentioned above. The course of all of the bands may be traced to the anterior tip of the body, as in *Microspironympha*; but the number of them originating at this point seems to be inconstant in the present species, and varies from

8 to 16. The direction of the spiral is dextrotropic, as in *Microspironympha*, and thus the opposite of that in *Pseudotriconympha*¹.

The surface of the body—covered by a well-developed periplast, as mentioned above—appears to be smooth, but good preparations reveal the existence of fine ridges running spirally in parallel with the bands of granules. The ridges and flagella in this organism are in close connexion, just as in the body of *Teratonympha*. The ridges are sharply pointed, though but little elevated, and are inclined strongly backwards, so that a shallow and narrow groove is formed behind each of them. The bands of granules are localized at the bottom of the grooves, and rather deep in the endoplasm, so that a small portion of the root of each flagellum is embedded in the protoplasm (Pl. XIII, fig. 53 b). The flagella are numerous and fairly long in this species, measuring 20–30 μ . Not only are the roots of the flagella embedded in the endoplasm, but their basal portions are also fixed on the surface of the body, just as in *Teratonympha*: they run backwards, lying fixed on the surface, until they reach the vicinity of the summit of the next ridge, where they become free. The ridges are commonly found depressed on the surface, so that the grooves behind them almost disappear and the characteristic structure is liable to escape the observer's eye.

The posterior end of the animalcule, somewhat metabolic as compared with the anterior, is destitute of spiral bands, as mentioned already, but is not naked. Here there are a large number of fine filaments, distributed thickly on the surface. They appear like cilia, but are distinguishable from the latter in several points. They seem brittle, less pliant than ordinary cilia, and slightly wavy. They are thickest at the middle portion and taper slightly towards both ends, and no structures such as basal granules are noticeable at their points of attachment. When observed in motion in the fresh state, they do not undulate together like ordinary cilia, but are swung independently in a peculiar manner, like fairly flexible filaments fixed at one end. It is possible that they are peculiar appendages like those described in *Parajoenia* by Janicki (1915), or they may be adherent micro-organisms, such as are often seen on other trichonymphids. They are slightly thicker and shorter than the flagella on the body wall. In the Brazilian species the posterior end, destitute of bands, seems narrower than in our forms; but in *H. hemigymnum* of Grassi, on the contrary, it is very wide. In these two species the posterior portion is apparently free from any appendages. Grassi described an area covered by "motionless cilia" (stereocilia) at the hind end of *H. mirabile*—his figure showing similar arrangements to those just described in *H. hartmanni*.

The greater part of the body of *H. hartmanni* is invested by a fairly tough periplast as described above. The endoplasm is specially compact under the body wall and is distinguished as a clear zone from the main portion, which is of looser constitution, and usually contains a large quantity of food débris.

¹ The spirals are drawn as right-handed by Grassi, and partly as right-handed and partly as left-handed by Hartmann—the latter being doubtless incorrect.

The nucleus is situated near the anterior end, embedded in a mass of special protoplasm, transparent, dense, and homogeneous, and staining somewhat deeply with eosin. In the young forms this peculiar protoplasm is only distinctly recognizable around the nucleus; but in large ones it is remarkably dense, and is also found strongly developed both in front of and behind the nucleus. In front it assumes the shape of a cone, reaching anteriorly to the tip of the body. At the sides and behind the nucleus, one, two, or three columns of it, gradually decreasing in thickness towards the hind end, are found hanging in the endoplasm (Pl. XIII, fig. 46). These columnar structures are remarkably distinct in some individuals and appear fibrous. Hartmann's description of the Brazilian species of *Holomastigotoides* contains no account of any corresponding structures, but quite similar structures are described and figured in *H. mirabile* and *H. hemigymnum* by Grassi. I am inclined to doubt the accuracy of Hartmann's observations on this point.

The nucleus of mature individuals appears usually elliptical in outline, measuring $20-26\mu$ by $10-15\mu$, the major axis being situated transversely. The nuclear membrane is very distinct, and the special protoplasm immediately surrounding it is dense. The internal structure of the resting nucleus is somewhat peculiar in this organism. In some individuals a chromatic body, resembling a band or a spindle and consisting of deeply stained granules embedded in somewhat deeply stained ground substance, is found crossing the nucleus transversely (Pl. XIII, figs. 43 and 45). The granules are, in some individuals, large in size and few in number, while they are minute and numerous in others. In some cases the chromatic body is divided into two halves situated side by side (Fig. 46). It is also common that the chromatic substance appears as a small number of globular masses or rod-like bodies, stained rather intensely and homogeneously with iron-haematoxylin (Fig. 44). The nuclear sap is not structureless and homogeneous as in the preceding forms, but fine granules, faintly stainable with iron-haematoxylin, are present in it. These granules are found, as a rule, apart from the chromatic bodies and distributed chiefly at the periphery of the nucleus. The situation of the chromatic bodies in the nucleus is noteworthy in this species. When they form a band or spindle, it lies transversely, as mentioned above, and is found commonly connected with the nuclear membrane at both its ends: when two masses are present, they are situated symmetrically at both sides, and they are also commonly kept in connexion with the membrane at symmetrical points. At the point of connexion of the chromatic bodies with the membrane a minute granule is frequently recognizable.

The process of division of the nucleus in this species appears somewhat peculiar. The nucleus, when going to divide, changes its position. It is drawn near one side of the body, and usually becomes narrowed towards the point drawn near the body wall. These changes are sometimes not so distinctly seen, or are unnoticeable because of the position of the nucleus under the microscope, but they are very conspicuous in some cases. In the nucleus

entering into division, the chromatic granules are small and numerous, embedded in the ground substance, and arranged in the form of a thick spindle (Pl. XIII, fig. 47). A short strand makes its appearance at the pole lying under the body wall, and the above-mentioned mass of chromatic granules is found hanging in the nuclear sap, attached to the nuclear membrane just at the place where the short strand is formed. The chromatin granules break up and become rearranged to form a certain number of looped threads with their ends attached to the ends of the strand. The strand and the threads now become gradually more and more distinct; the strand becomes thickened and elongated, though rather slightly, and the threads become thick and uniform chromosomes (Pl. XIII, figs. 48 and 49). In early stages there are usually two long threads and some shorter ones; the former assume the form of U- or V-shaped loops, fixed at both ends of the strand, while the latter are found hanging from either end. The former threads are those that persist as chromosomes through the later stages. I have counted one to three additional threads in nuclei at a rather advanced stage in division, but they disappear later. The chromosomes having the shape of a loop divide transversely into two at the middle, and each limb becomes a daughter chromosome. Pl. XIII, fig. 50 represents one of the specimens in the most advanced stage which I could study. In this stage the strand measures 4–5 μ , and is fused with the nuclear membrane. It is fairly thick, pointed at both ends, and stained deeply with iron-haematoxylin, showing occasionally a fibrous structure. The individual shown in Pl. XIII, fig. 51 is also in the most advanced stage, and the daughter chromosomes are distinct, but the strand is invisible, owing to its disposition. The next stage which I have been able to find shows the already completely separated daughter individuals. I could not follow accurately the later stages of division. It will not be unreasonable, however, to suppose that the strand becomes further elongated, as in the preceding genera; but I am inclined to suppose that the nucleus is quickly divided after the above stage is reached.

In young animalcules lately divided, the nucleus is round and the two daughter chromosomes are found hanging side by side from the anterior pole. In many cases, the greater portion of the chromatin becomes, sooner or later, aggregated into an oval mass, hanging in the nuclear sap at two points, and then disintegrates, forming the characteristic structure of the resting nucleus, with a rather feebly stained ground substance containing intensely stained granules embedded in it (Pl. XIII, fig. 52). As the animalcule grows, the nucleus becomes gradually flattened and elliptical in outline, and the points of attachment of the chromatic body become separated gradually from each other. Parallel with the alteration of the position of the points of attachment to the membrane, the shape of the chromatic body becomes also altered so as to form a band or spindle, which may be divided into two halves. In some cases, the daughter chromosomes remain in a rod-shaped condition for a long time; or they may become divided into two, three, or four pieces, and persist in that condition.

Hartmann's statements regarding the division processes of the Brazilian species appear very doubtful to me. Basing his opinion upon a small amount of material, he concluded that both amitosis and mitosis may occur. As for the latter, he figures several stages interpreted as prophases, in which chromatin granules are distributed in the nuclear sap in the form of irregular threads. They differ strikingly from the prophases of mitosis in our forms. As for the amitosis, he described two stages, one interpreted by him as an early stage, and the other as a young form just after division. Specimens with the nucleus of the same type as that interpreted by him as an early stage of mitosis are frequently met with in our species; but these are merely forms with resting nuclei of a special type, as mentioned above, and have nothing to do with division. The forms interpreted by him as the young have a structure so remarkably different from the parental form, that no one can recognize their genetic connexion, in the absence of intermediate forms. Hartmann, however, neither discovered any forms showing an intermediate structure nor gave any evidence to indicate their genetic connexion. I have failed to find any forms with a similar organization in the Japanese termites.

VI. *Spirotrichonympha*.

The genus *Spirotrichonympha* was introduced by Grassi (1911) for the flagellate originally wrongly referred by him (1892, 1893) to Leidy's genus *Pyrsonympha*. The type species of this genus is *Sp. flagellata*, described by Grassi in his work with Sandias (1893). It has since been renamed *Leidya metchnikovi* by França (1916). (See França, 1918, and Grassi, 1917.) In his recent work, Grassi (1917) distinguishes three species of this genus; one of them with two varieties. These are *Sp. mirabilis* (in *Porotermes adamsoni* from Australia), *Sp. flagellata* var. *schedorhinotermis intermedii* (in *Schedorhinotermes intermedius* from Australia), *Sp. flagellata* var. *coptotermis lactei* (in *Coptotermes lacteus* from Australia), and *Sp. elongata* (in *Schedorhinotermes intermedius*). In my former paper (1917), I described an organism harboured by *Coptotermes formosanus* under the name of *Cononympha leidy*, establishing a new genus for it, because I could not identify it as a species of *Spirotrichonympha*, the characters of which genus were given very briefly in Grassi's work of 1911. But his recent work made me clearly understand that my organism belongs to this genus. The Japanese species appears to differ, however, from all species reported.

Spirotrichonympha leidy sp. nov. (Plate XIII, figs. 41 and 42).

This is the smallest of the forms found in *Coptotermes formosanus*, and measures 15–50 μ in length and 8–30 μ in breadth; that is to say, the largest individuals hardly exceed in size the smallest forms of most other species found associated with them. This organism is also clearly distinguishable by its shape from the other forms in the same host. It has usually the shape of a

tall cone, the posterior or basal part of which is slightly convex. The body wall is more or less rigid at the sides, chiefly owing to the character of the bands of flagella described below, only the posterior basal part being somewhat metabolic.

Spiral bands of deeply stained granules, containing the basal granules of the flagella—such as are seen in *Microspironympha* and *Holomastigotoides*—are found over the greater portion of the surface. The number of the bands appearing on one side varies from 10 to 20, and their course is rather oblique: but this does not represent the true number present, which I have not yet succeeded in determining. The disposition of the bands is peculiar in this genus: they are not found directly under the periplast, but are situated somewhat more deeply in the protoplasm, a portion of the root of each flagellum being embedded in it. The above character is especially marked at the anterior end, and becomes less distinct towards the posterior; that is to say, the basal granules of the flagella lie deeper at the anterior end of the body. The flagella are fairly long, measuring 10–16 μ , and run rather sharply backwards. The direction of the spiral of the bands is the same as in the preceding genus, namely dextrotropic or right-handed; that of the forms studied by Grassi is drawn also as right-handed by him. The posterior portion of the body is free from any appendages.

The nucleus is oval, and is found in the middle region of the body. Its internal structure is of a type resembling that of *Pseudotrichonympha*, consisting of clear and structureless ground substance and chromatic bodies arranged in an irregular network or scattered under the nuclear wall and in the ground substance. One of the most characteristic features of this form is a conical structure at the anterior region of the body, consisting of dense and homogeneous protoplasm, and apparently of the same substance as the particular protoplasm surrounding the nucleus in *Holomastigotoides* and *Microspironympha*. It appears most dense in its consistency and most distinct in its outline at the anterior part, becoming gradually less dense and less distinct towards the posterior. This mass of protoplasm is almost invisible near the nucleus, but I think it probable that it there merely becomes thinner, and the nucleus is embedded in it.

As has been pointed out by Grassi (1911 and 1917), the grounds for Hartmann's view that a form closely resembling this was the young of "*Trichonympha hertwigi*" were certainly insufficient. As regards the genetic connexion of his "young form" and "male form," he himself said that all intermediate types were not made out—especially as regards the arrangement of the flagella, no intermediate forms having been found. As regards the structure of the anterior end, he remarked that some intermediate types were observed, but neither descriptions nor illustrations of these forms were given in his paper. In our organisms, no forms showing characters intermediate between this form and *Pseudotrichonympha* were met with: and as the rows of flagella of *Spirotrichonympha* are in dextrotropic spirals, while those of *Pseudotrichonympha*

are laeotropic, it is hard to imagine that the one can grow into the other. As for the relationship between his "young form" and "female form" (*Holomastigotoides*), there are some points of resemblance in their organization. But they differ distinctly in the disposition of the basal granules of the flagella and the structure of the nucleus. No intermediate forms were recognizable, and the existence of a genetic connexion between the two is hardly conceivable.

Although *Spirotrichonympha leidy* occurs in all the individuals of *Coptotermes formosanus* which I have examined, it is not so abundant as some of the other species. Unfortunately I have been unable to find any dividing forms, or other stages in development.

VII. *Holomastigotes*.

This is a genus established by Grassi (1892), and described in his work with Sandias (1893), for a small organism found in Italian termites. He defined the genus more accurately later (Grassi, 1911) and published further details and figures in 1917. Forms closely resembling or probably identical with it are also found among the figures of the American trichonymphids, given by both Leidy (1881) and Porter (1897), but erroneously regarded by them as possible young stages of *Trichonympha*. An organism described and figured by Comes (1912, Fig. 1) as a "young *Pyrsonympha* with resting nucleus" also appears to belong to this genus.

Closely similar forms also occur in the species of *Leucotermes* of Japan proper and of Formosa. The shape is variable in our organisms, and no sharp morphological distinction can be drawn between them and the Italian or the American forms. As regards the finer details of structure, it is not possible to determine whether they show any differences or not, owing to the insufficiency of previous descriptions. I propose, therefore, to call our form provisionally by the name of the Italian species described and figured by Grassi (1917) in his recent work.

Holomastigotes elongatum Grassi (?) (Plate XIII, figs. 39 and 40.)

This is a small and commonly fusiform or lanceolate form, measuring 15–45 μ in length and 10–15 μ in breadth. According to Grassi (1893) the type species attains a length of 70 μ and a width of 24 μ . The forms which I have studied thus appear to be considerably smaller, and should therefore, perhaps, be regarded as an independent variety. Grassi (1917), however, in his recent work does not state the dimensions of his species; but from his figures, of which the magnification is recorded, it appears that the organism may be considerably smaller than his original account would lead one to suppose. The body usually tapers towards the posterior end, and the anterior end usually projects slightly, forming a mammiform eminence. In this feature it appears to differ from the forms described by Grassi. The animalcule is, however,

variable in form, and, when alive, is frequently club-shaped or pear-shaped.

The periplast is fairly well-developed and distinct ridges are found on the body wall, winding spirally from the anterior tip to the posterior end. The direction of the spiral is also right-handed in this form¹. The edge of each ridge seems rather sharp and stiff, especially in the anterior region, so that it appears as though supported by a cord. The number of ridges seems to be eight. They are sloped backwards and at the posterior edge of each of them a shallow but distinct groove is formed behind; also a distinct row of basal granules of the flagella is there found, embedded in clear protoplasm. These spiral ridges are thus identical with the spiral ridges in *Holomastigotoides*, and represent the same type of structure as is seen in the well-developed transverse ridges in the body wall of *Teratonympha*. The disposition of the flagella is also identical with that of *Teratonympha* and *Holomastigotoides*. The basal end of each flagellum is adherent to the surface of the ridge and becomes free at its edge, so that the surface of the body between the ridges or folds appears beautifully and densely striped (Pl. XIII, figs. 39, 53 c). The free portion of the flagellum measures 10–15 μ . The endoplasm is rather clear, and usually contains a large number of small refringent globules ("round corpuscles" of Grassi), taking haematoxylin and eosin somewhat deeply (Pl. XIII, fig. 40). It is peculiar to this form that wood particles and other food débris are not usually present in the endoplasm.

The nucleus is situated at a short distance from the anterior tip, and is usually pear-shaped. There is a mass of peculiar dense protoplasm, as seen in the preceding genera, at the anterior tip of the body, and the nucleus is usually found embedded in it. The internal structure of the nucleus is somewhat peculiar in this form. It consists of a structureless ground substance, staining rather deeply with iron-haematoxylin, and having several chromatin masses embedded in it.

I have found no other stages of development than that just described, and I have encountered no dividing forms in my preparations.

(2) *Pyrsonympha* Series.

This group embodies a number of forms clearly distinguished from the members of the previous series. In them the body is not invested with numerous flagella resembling cilia, but is provided with peculiar slender flagellum-like cords, four or eight in number, which I propose to call "flagellar cords" ("undulating lines" of Leidy, and "contractile cords" of Porter). It is true that, in some forms, delicate appendages are found distributed over the body, giving it the appearance of being invested with cilia; but these appendages are quite different in nature from ordinary cilia or flagella, and are something like those seen at the posterior end of *Holomastigotoides* and

¹ Drawn right-handed by both Grassi (1917) and Porter (1897, Plate III, figs. 30–32, 35, 36).

in some other trichonymphids. Some of them are doubtless adherent micro-organisms, but some are probably peculiar appendages similar to those described in other Protozoa by such names as "bristles," etc. One other characteristic structure of the members of this group is an elastic filament, which I shall call the axial filament ("undulating cord" of Leidy and "flagellum" of Porter), lying in the protoplasm and stretching from the anterior tip of the body to the posterior end or its vicinity. The flagellar cords arise at the anterior tip of the axial filament and run spirally backwards, attached to the body wall, and become free at the posterior end. The direction of the spiral is always left-handed (laeotropic). Porter described the direction of the spiral of the cords in the American species as right-handed, but the figures given by him are drawn as left-handed. I think it probable that his description is a mistake, and his figures are correct. The drawings of Grassi are left-handed¹.

This group comprises two genera of the American authors, *i.e.* *Pyrsonympha* and *Dinenympha*, two of the three oldest genera established for the Protozoa of American termites by Leidy in 1877. One species was described by him under each of these genera, and figures were given in his later work (1881). The same names were applied by Porter (1897) to similar forms, studied by himself in the same country. Grassi (1893), in his work with Sandias dealing with the Italian termites, described two species which he referred to the above two genera. The form referred by him to the genus *Pyrsonympha* is one thickly covered with flagella, differing distinctly from the type of the genus described by the American authors. It is thus unquestionable that he made an error in the application of the generic name. He has himself corrected this mistake in later publications (1911, 1917); and the organism which he originally called by Leidy's name is now the type species of the genus *Spirotrichonympha*, established by him (Grassi, 1911). The forms described by Grassi (1893) under the name of *Dinenympha gracilis* differ also from the forms to which Leidy gave that name, and represent several types with the characteristics of both *Pyrsonympha* and *Dinenympha* of the American authors. Originally Grassi did not draw any distinction between Leidy's two genera; but it appears that he is now of the opinion that the type forms of the two genera are merely different stages of growth of a single form; and in a review of the genera given in his work which appeared in 1911, the two were united into one genus, *Dinenympha*. Comes (1910 *a*) seems to be of the same opinion as Grassi, for he put forward the view that various forms referable to Leidy's two genera represent different stages of development of *Dinenympha gracilis*. The organism which he described as "*Pyrsonympha*" subsequently (1912) is in reality *Spirotrichonympha* and *Holomastigotes*. However, he has also described an organism closely similar to Leidy's *Pyrsonympha*, referring it to the new genus *Lophophora* (1910). Grassi (1911) regards this as a stage in development of the same organism, and refers it

¹ Grassi and Sandias (1893), "*Dinenympha gracilis*," Plate V, figs. 11-14.

to the genus *Dinenympha*. Recently Zulueta (1915) has published a paper on "*Dinenympha gracilis*," but the organism which he describes is clearly the same as that which Leidy called *Pyrsonympha*.

In *Leucotermes* of both Japan proper and Formosa, forms belonging to this group are found in large numbers. They are so numerous that they far exceed the members of the preceding group in number. They present such remarkably diverse types of organization that it is clear they cannot be referred to one or two species, as has been done by previous authors in the case of the American and Italian forms. Some of our forms are of such peculiar organization that they are easily distinguishable from each other, but there are several which are not so readily distinguishable, and their classification demanded much time and work. I finally determined that they are to be referred to eight species—one of them doubtful.

Previous authors do not seem to have been sufficiently accurate in classifying their species. The illustrations given by the American authors are more or less detailed; and they show clearly that several different forms, which undoubtedly present characteristics proper to distinct species, are included under a single specific name. It can be clearly recognized, moreover, that the forms referred by Porter (1897) to *Dinenympha* are of a different character from the original forms described under this name by Leidy. As for the works of Grassi (1911), Comes (1910, 1910 *a*, 1912), and Zulueta (1915), both their descriptions and illustrations are so incomplete that the identity of their forms with the others is hardly ascertainable.

The classification of the organisms of this group described hitherto is thus in a very confused state; and this has puzzled me very much in the adoption of specific names. I have finally been compelled to call all the species occurring in the Japanese termites by new names.

Our forms may be divided into two groups, which I think to represent the characteristics of *Pyrsonympha* and *Dinenympha* of Leidy. The differences found between these two groups, however, were not thought to be so distinct as to justify their reference to different genera: so I have marked the distinction by classifying them as two subgenera of a single genus. Thus I agree with Grassi (1911) in the conception that Leidy's two genera should be united into one, but I cannot concur with him in the adoption of the generic name. I propose to adopt *Pyrsonympha*, not *Dinenympha*, as the generic name, for the following reasons: the characters of the group are more typically and distinctly represented in *Pyrsonympha* than in *Dinenympha*, and the former name stands prior to the latter in the original description of Leidy (1877). I agree with Grassi (1911) that *Lophophora* Comes is a synonym: and I may point out that this name cannot be used in any case, as it is already preoccupied—having been proposed on at least two previous occasions for insects.

The chief points of distinction of the two subgenera are as follows:

I. **Pyrsonympha**. The body very large, large, or medium sized; piriform, club-shaped, or screw-like. The body is twisted and the flagellar cords wind round it; some forms being twisted into a screw, but not curled, as a whole, into a spiral. The axial filament hangs in the endoplasm, ending freely in it at the posterior end. Two species are distinguished:

- (1) *P. grandis* sp. nov. (in *Leucotermes speratus* and *L. flaviceps*).
- (2) *P. modesta* sp. nov. (in *Leucotermes speratus* and *L. flaviceps*).

II. **Dinenympha**. The body small and slender; club-shaped or lanceolate. Whole body curled into a spiral. The axial filament distinct or indistinct: in the former case its posterior end is held fixed at the posterior tip of the body. Five species and one doubtful species are distinguished:

- (1) *P. (D.) exilis* sp. nov. (in *Leucotermes speratus* and *L. flaviceps*).
- (2) *P. (D.) rugosa* sp. nov. (in *Leucotermes speratus*).
- (3) *P. (D.) nobilis* sp. nov. (in *Leucotermes speratus*).
- (4) *P. (D.) leidy* sp. nov. (in *Leucotermes speratus* and *L. flaviceps*).
- (5) *P. (D.) parva* sp. nov. (in *Leucotermes speratus* and *L. flaviceps*).
- (6) *P. (D.) porteri* sp. nov. (?) (in *Leucotermes speratus* and *L. flaviceps*).

I. *Pyrsonympha*.

The organisms belonging to this subgenus vary remarkably in size, and their shapes are, moreover, somewhat conspicuously changeable. Thus I have been greatly puzzled in determining the exact number of species to which our forms are to be referred. As a result of protracted observations, I have arrived finally at the conclusion that there are really two sets of forms, each set referable to an independent species. Both of these species are common in *Leucotermes speratus* of Japan proper and *Leucotermes flaviceps* of Formosa.

- (1) *Pyrsonympha grandis* sp. nov. (Plate XIV, figs. 54–64).

The forms of this species, like the others of the same subgenus, differ from the members of the preceding series in their mode of life in the intestine. They do not always live freely in the lumen of the gut, but a large number of individuals are found attached to the wall and hanging in the cavity. The forms attached to the wall are usually so numerous that they far exceed the unattached forms in numbers. They are found closely assembled and hang perpendicularly from the intestinal wall, assuming the shape of a club, rather long and straight or slightly curved. In individuals living freely in the lumen, the body is shortened and thickened, especially at the posterior region, so as to assume the shape of a flask, with a rather large body and a somewhat slender neck. Frequently the organism is so thickened that the body becomes piriform; but sometimes it is rounded anteriorly, so that the body appears oval. The same mode of life was noticed by Porter in the American species. According to him, the young forms are found attached to the intestinal wall

and the mature ones invariably swim freely in its cavity. It is also the case in our forms that most individuals attached to the wall appear to be small, but attached individuals of large size are also found quite commonly. It is by no means rare, moreover, to find small forms swimming freely in the cavity of the gut. In the club-shaped forms hanging from the wall, the body is almost straight or slightly curved, but the course of the flagellar cords on the body wall is distinctly spiral, indicating clearly that the body is twisted in various degrees. In the forms living freely in the intestinal cavity, the body is not only twisted but also spirally wound as a whole. Forms hanging from the wall do not remain quiet, but continue to move gently. Free-swimming forms usually display vigorous motions; and though they do not change their position markedly, the anterior portion is commonly swung very actively in all directions.

The dimensions also vary considerably in this species. Individuals measure usually 40–150 μ , occasionally as much as 170 μ , in length, and 19–40 μ , sometimes up to 50 μ , in breadth. According to Leidy (1881), *Pyrsonympha vertens* measures 100–160 μ in length, but Porter describes specimens attaining a length of 275 μ and more. Comes (1910 *a*, 1912) and Grassi (1893, 1911) give no measurements of their forms, whilst Zulueta (1915) states that his species (called “*Dinenympha gracilis* Leidy,” but really a *Pyrsonympha*) measures from 18 μ to 90 μ or more.

The axial filament is very well-developed in this species. It hangs from the anterior end of the body in the endoplasm and lies quite free from the body wall throughout its entire length. It is thick, and oval or elliptical in cross section at the anterior portion. Towards the posterior end, it becomes gradually thinner and slender. Its posterior extremity is not connected with any part of the body wall, but ends freely in the endoplasm. In young individuals, the filament is slender and stains homogeneously with iron-haematoxylin and also with eosin. In large forms, however, it is thick at the anterior portion, and the posterior is usually found split into two, three, or sometimes more, slender filaments (Pl. XIV, figs. 54 and 55). The anterior portion of the thick filament is commonly not homogeneous, and a deeply stainable wall and somewhat feebly stainable contents are distinguishable, though the boundary between these parts is indistinct. In some individuals a small portion at the anterior extremity shows an appearance as if consisting of several slender cords, taking dyes intensely, arranged regularly on the surface of a rod staining very faintly (Pl. XIV, fig. 57).

As regards the axial filament, some differences are recognizable between our forms and the American ones. The filament does not end freely at its posterior extremity in the American forms, but is fixed at the hind end of the body; and it seems, moreover, to remain simple, never splitting even in the later stages of growth.

In *Pyrsonympha grandis* the anterior extremity of the axial filament is more or less abruptly pointed, and it is at this pointed extremity that the

characteristic flagellar cords arise. No special structure such as a basal granule is recognizable at the point of connexion of the cords and the filament. In the majority of cases the pointed extremity of the filament is found at the anterior tip of the body, and the cords arise directly from it at that point; but not unfrequently the pointed end of the filament lies a little within the endoplasm, and is then provided with a rather thick stalk connecting it to the anterior tip of the body, the cords arising from the end of the stalk (Pl. XIV, fig. 57). The number of the cords is four in the young forms and eight in the mature ones. They run backwards, attached to the surface of the body, to the posterior end, where they become free. Porter remarked that the cords of the American species "can be traced to the posterior extremity of the animal, and thence back again on the opposite side of the animal to the anterior end." This is not true of our forms. The free ends of the cords, on the contrary, are fairly long and distinct, and never escape the eye of a careful observer. The cords run in a left-handed spiral round the body. They are rather straight in small forms, while they are remarkably wavy in large forms, and the larger the body, the more conspicuous is their winding. The surface of the body is not even, but each cord is situated on a ridge. In small forms the ridges are rather indistinct; but they are distinct in large forms, especially at the anterior end, where they frequently appear as rather flat and elevated membranes resembling the undulating membrane of *Trypanosoma*. In the living animal the cords and their membranes display a continuous and beautiful rippling motion, the ripples passing from before backwards.

In the majority of forms, the body is naked and destitute of any kind of appendages, but rarely large individuals invested rather thickly with filamentous bodies are met with. In the American species studied by Leidy some are naked, while others are thickly "ciliated," and those studied by Porter seem to be invested thickly without exception. It seems certain that the filamentous appendages seen in our forms are not real organs of the animals themselves.

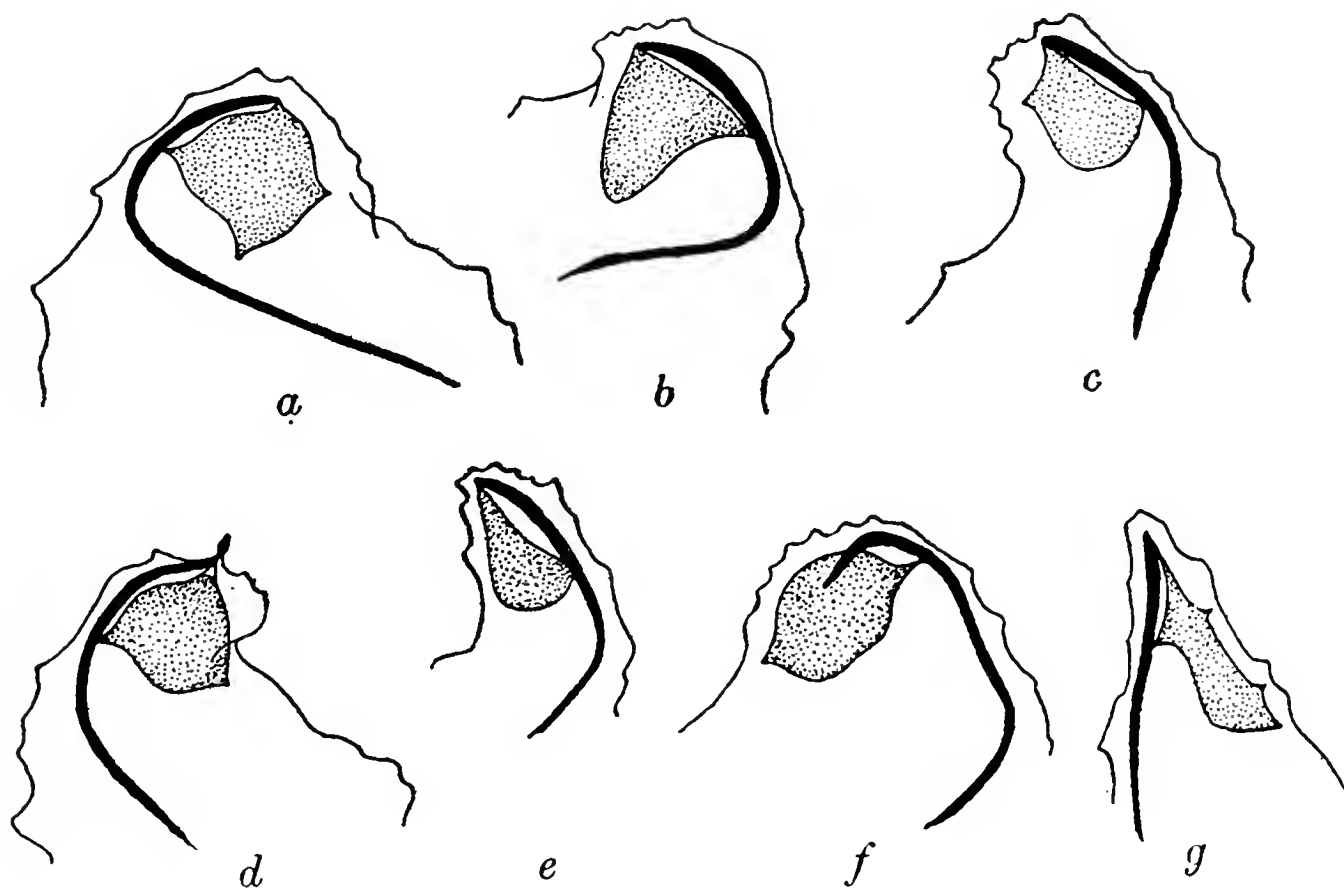
Individuals lately detached from the gut wall are frequently provided with a knob-like structure, stained quite as deeply as the axial filament, at the anterior end of the body, where the pointed end of the filament is fixed. The knob is usually situated at the tip of the axial filament, but sometimes a short peduncle connecting them is observable. In some forms the knob assumes the shape of a sphere or an ellipsoid, and is unbroken in its outline (Pl. XIV, fig. 54); but in others it is not smooth but prickly at its distal end (Pl. XIV, figs. 57 and 62). Long and distinct splinter-like filamentous structures are also frequently visible at this end (Fig. 56). This split or frayed-out structure is variable in size and shape; it is commonly single, but sometimes two or more are found. As regards the function of the knob, I am of the opinion that it serves, when the animalcule is attached to the layer of chitinous substance investing the inner surface of the intestine, to preserve a firm connexion.

It seems highly probable that the knob itself is made of the substance of the axial filament. According to Porter's description, there is, in the American forms, a knob and a long filament ("peduncle" in his terminology), and the latter is inserted deeply into the intestinal wall. Sections of the intestinal wall of our termites were carefully studied, but I failed to recognize the existence of any structure corresponding to the "peduncle" of Porter. The splinter-like filaments in our forms are no other than artifacts, being produced artificially in preparing the animalcules for examination; and they are undoubtedly merely fibrous fragments or strips of the chitinous lining of the intestine. Similar structures to those just described have been observed in a species of *Pyrsonympha* by Comes (1910), but his interpretation of them is very different. He regards the frayed-out chitinous end as a tuft of consolidated flagella, and the knob as a blepharoplast: and proposes on account of this peculiar condition of the "flagella" to place the organism in a new genus *Lophophora*. As already noted, I agree with Grassi (1911) that this name should be regarded as a synonym of *Pyrsonympha* (*Dinenympha* of Grassi).

The nucleus is rather large and is situated at the anterior end of the body. It does not lie freely in the endoplasm but is closely connected with both the axial filament and the body wall, so that its shape is subject to modification, according to the state of contraction or extension of the anterior portion of the body. In the small club-shaped forms with slender bodies, the nucleus is also club-shaped and lies closely along the axial filament (Pl. XIV, fig. 54). In the large sized forms the nucleus is piriform, or rather triangular in outline, invariably much narrowed, or pointed, at the anterior pole. The nucleus becomes triangular, quadrangular, or semicircular in outline when the anterior part of the body is thickened. The anterior pole of the nucleus is found at the anterior tip of the body or in its vicinity, and it can be clearly seen in many organisms that the nucleus and the axial filament are kept in close connexion at their anterior ends. In many cases, the contour of the nucleus is smoothly rounded, except at the anterior pole; but in individuals abnormally thickened anteriorly it appears peaked at a few other points, and then it can be clearly seen that it is not only joined to the axial filament at another point, but also at one, two, or sometimes more points to the body wall. In Text-fig. C, *a-g*, several forms showing the modes of attachment of the nucleus are depicted. In the majority of cases, the peaked points of the nucleus touching the axial filament are directly attached to it. But in exceptional cases, the pointed anterior pole of the nucleus is found somewhat separated from the tip of the filament and connected with it by means of a thread. Once I met with a specimen in which the nucleus was rounded at its anterior tip, and connected with the tip of the filament by a funnel-like membrane (Text-fig. C, *d*). It thus seems probable that it is not the nuclear membrane itself that comes in contact with the filament, at least at the anterior pole, but that there is a membranous structure or sac, which invests the nucleus very tightly, and is kept in connexion with the axial filament and the body

wall at several points. As remarked above, the number of peaks visible on the nucleus is variable, depending on the condition of the body, and it is doubtful whether the points of attachment of the nucleus are constant in position or not.

The position and the shape of the nucleus seem to be quite different in the American and Southern European species of *Pyrsonympha*. In these the nucleus assumes an oval or an elliptical shape, and is situated away from the anterior tip of the body and not so closely connected with the axial filament. No description of the attachments of the nucleus to other parts of the body are to be found in the works of previous authors (Leidy, Porter, Grassi, Comes, and Zulueta). Some of the figures given by them, however, seem to indicate the existence of a structure similar to, but less developed than, that of our forms.



Text-fig. C. Anterior portion of *P. grandis*, showing various conditions of the axial filament and the nucleus. [Drawn with camera lucida. ($4 \times \frac{1}{12}$.)]

The internal structure of the nucleus of *Pyrsonympha* is quite different from that of the trichonymphids. It is of a type resembling that of the gregarines and coccidia, consisting of a fine and distinct achromatic network with minute chromatin granules, and one, two, or more large chromatic bodies, or karyosomes, situated centrally and usually surrounded by a clear space. The chromatic body has a vacuolate structure, and closely resembles the karyosome of gregarines and coccidia, but is often somewhat rough in its contour (Pl. XIV, figs. 54–56).

The endoplasm appears more or less homogeneous and somewhat compact in consistency, and rather closely resembles that of *Microspironympha* and *Holomastigotes*. In the small forms the endoplasm appears rather clear; but

in the large individuals it is frequently vacuolated, and great numbers of globular bodies of a peculiar kind are commonly found in it. These globules are rather refractive in fresh specimens, and stain with eosin somewhat deeply, but faintly with iron-haematoxylin¹. As in many members of the preceding group, food débris is usually present in the protoplasm, but consists not only of vegetable matters (wood, etc.) but also of the bodies of other animalcules, such as small individuals of *Dinenympha*. Animalcules ingested into the endoplasm are found rounded and shrunk into spherical masses, and various stages of their disintegration, probably due to digestion, can be recognized.

Dividing forms were met with very rarely in this species, and I was barely able to make out the chief stages of the process, after an eager search through large numbers of termites in both Japan proper and Formosa. Division seems to take place invariably in the forms living freely in the lumen of the gut. In individuals going to divide, the body becomes rounded and the axial filament detached from both the nucleus and the body wall and set free in the endoplasm. The nucleus becomes oval or round in shape, and probably separated from the body wall, except at its anterior pole, so that it remains attached at only this one point, from which the flagellar cords arise. The nuclear network now disappears, the karyosome breaks up, and globular masses of chromatin make their appearance scattered in the almost structureless nuclear sap (Pl. XIV, fig. 58). Thick rod-shaped chromosomes are now formed from the chromatin, and a mitotic figure, somewhat different from that seen in the trichonymphids but resembling rather closely that of metazoan cells, makes its appearance. No structure corresponding to the strand seen in all forms of the preceding series is visible; but a distinct achromatic spindle is formed, with the chromosomes arranged at its equator (Pl. XIV, fig. 60). The chromosomes divide transversely, and the daughter groups move towards opposite poles in due course. The nuclear membrane persists throughout the entire process, and minute centrioles can be made out at the poles of the spindle in the later stages.

The flagellar cords remain connected with the nucleus, four of them being found attached to each pole of the spindle (Pl. XIV, fig. 61). Once I noticed a short fibrous strand on the membrane of the nucleus in an early stage of division (Pl. XIV, fig. 59). Unfortunately I have been unable to ascertain its origin and fate, or to determine its relation to the ends of the cords; but I am inclined to believe that it represents a stage in the formation of the achromatic spindle.

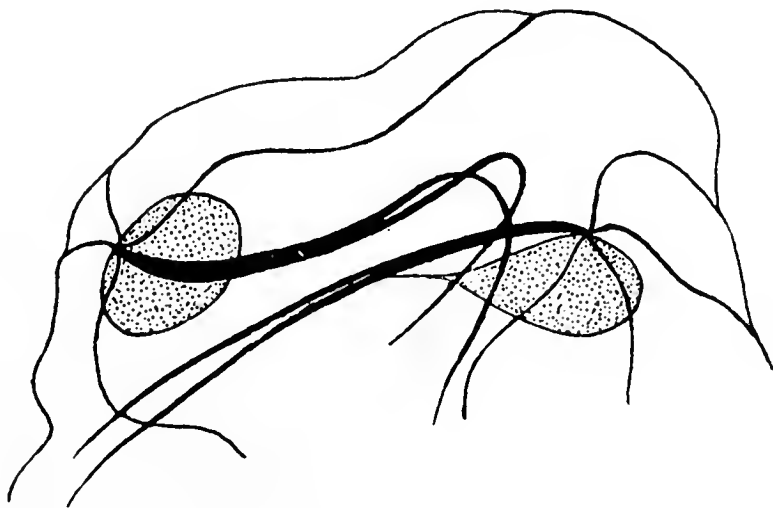
The axial filament gradually degenerates in the endoplasm and disappears before the division of the nucleus is completed. Shortly after the daughter nuclei are formed, two new axial filaments can be seen, one attached to each of them (Text-fig. D). As regards the origin of the new axial filaments I have

¹ The vacuoles are usually present in only the posterior two-thirds of the body. It was the presence of such vacuoles which apparently led Comes (1910) to bestow the specific name *vacuolata* upon the *Pyrrsonympha* which he regarded as belonging to a new genus *Lophophora*.

nothing to say with certainty. At the time when they first become visible in my preparations, no centrioles can be seen at the points where the flagellar cords join the nuclear membranes.

I have found no other processes of multiplication than those just described. The stages of division of *Pyrsonympha* (incorrectly called *Dinenympha gracilis*) described and figured by Zulueta (1915) agree closely, so far as they go, with those which I have observed. On the other hand, the remarkable cycle of development described by Comes (1910 *a*, 1912) in similar forms is very different from anything that I have seen. This author describes "males" and "females," "conjugation," "multiple fission," and a peculiar development in the salivary glands of the termites. I agree with Grassi and Zulueta in believing that no such stages exist, and that Comes' interpretation of his findings is incorrect.

As mentioned already a remarkably large number of individuals are found attached to the intestinal wall: and these are not only young and small forms,



Text-fig. D. A late stage of division of *P. grandis*, showing the flagellar cords and newly formed axial filaments. Drawn with camera lucida. ($4 \times \frac{1}{12}$.)

but large and mature ones as well. In studying these forms I often noticed gigantic piriform or fusiform individuals with a conspicuously jagged surface, distinguishable at a glance from the normal forms. On making a special search for these peculiar forms in a large number of termites, I was able to find not a small number of them, representing several types of structure. At a time when I had insufficient material, I was inclined to imagine that they represent stages in a particular mode of multiplication. The fact that a large number of young forms are found hanging from the wall in close association seemed to favour this supposition. But on studying more material, I soon realized that they represent successive stages in a process of physiological degeneration.

In the early stages of degeneration the nucleus becomes detached from both the axial filament and the body wall; in some forms it is broken in two, and one half remains connected with the filament at its normal position, while the other is set free in the endoplasm (Pl. XIV, fig. 62). The endoplasm itself

appears denser, but contains no food débris, while a large number of minute globular bodies, staining deeply with eosin and faintly with iron-haematoxylin, are found scattered in it. The nucleus, suspended in the endoplasm, becomes gradually hypertrophied and disintegrated. The axial filament also disintegrates by a somewhat peculiar process; it does not become detached from the anterior end of the body—as it does during division—but remains fixed in its normal position and becomes strongly curled into a rather regular spiral (Pl. XIV, fig. 63). In degenerating nuclei the network becomes indistinct, until finally it vanishes entirely. The karyosomes break up into irregularly shaped and ill-defined masses of chromatin, isolated from each other or fused together. In Fig. 64 is shown a nucleus at an advanced stage of degeneration, with several clumps of chromatic substance, stained rather faintly with haematoxylin, embedded in a coarsely granular ground substance. The clumps stain more faintly and their contour gradually becomes less distinct. Hand in hand with the above changes, the nuclear wall becomes thinner and the globules in the endoplasm increase in number, especially in the vicinity of the nucleus. The filament also becomes gradually indistinct, and soon disappears, apparently dissolving in the endoplasm.

The process of physiological degeneration has been more or less thoroughly investigated in artificially cultivated individuals of several forms of free-living Protozoa by R. Hertwig, Prandtl, and others. As regards tissue-parasites, there are also some descriptions by Schaudinn and Léger of degeneration observed in *Coccidia*. Regarding the other intestinal Protozoa, however, few accounts have hitherto been given. Moreover the process in *Pyrsonympha* shows some interesting peculiarities. How is it that such an uncommon phenomenon is seen in this species? This is a question of considerable interest, and I am of the opinion that the answer is to be sought in certain peculiarities both of the protozoon itself and also of the intestinal wall of its host. In the other forms leading a free-swimming life in the cavity of the gut, individuals inclined to degenerate cannot long remain in the intestine, but sooner or later are cast out. In *Pyrsonympha*, however, the body is firmly united to the intestinal wall, and the mode of attachment is peculiar. It is not a cell of the intestine, but its covering layer of chitin, to which the animalcules are attached; and it is not by means of the soft part of the body, but by a special rigid structure, that the connexion is maintained. The attachment is thus purely mechanical, and does not depend upon the vital activity of the flagellate. It seems, therefore, highly probable that the animalcules may remain fixed in position, regardless of their state of vitality; so that if they die or degenerate, they do not pass out of the body, like the forms living freely, but remain, though dying and even dead, still suspended from the intestinal wall.

(2) *Pyrsonympha modesta* sp. nov. (Plate XIV, figs. 65 and 66).

The individuals of this species resemble the young forms of the preceding, but show some differences in their shape and some other structures. They are fairly small in size, measuring 30–80 μ in length, and are commonly pear-shaped. The nucleus is oval or round, and is situated at the anterior end. The flagellar cords run almost straight, and the ridges on which they are situated are commonly indistinct, except in much twisted forms. The cords themselves are confined, at the anterior end, to one side of the body, the other being free from them. The axial filament is more slender than in any individuals of the preceding species, and is always single. The endoplasm is usually clear, though it frequently contains globular bodies. A peculiarity of this form is that the body is often conspicuously twisted, with the anterior end bent back into a peculiar form, so that it resembles a screw (Pl. XIV, fig. 66). In such individuals the bare area—noted above—at the anterior end is seen to lie in the inner angle of the bend.

Dividing forms are also rare in this form, and the process of division appears, as far as I could conjecture from my material, to be of a type identical with that of the preceding species.

II. *Dinenympha*.

This subgenus embodies a number of species, distinguished from the preceding forms by the shape of the body and the mode of arrangement of the axial filament. The body is slender and usually assumes the shape of a club or an elongated spindle, nearly oval in its cross-section: or it may be rather flattened and ribbon-shaped, showing more or less distinct edges at both sides. The body is twisted and spirally wound in a characteristic manner, showing two to five undulations as seen from the side (commonly two or three, but four or five in some species). The body is less metabolic than in *Pyrsonympha*, though abnormally thickened and shortened forms are met with rarely in some species. Such individuals may resemble small forms of *Pyrsonympha*, but are easily distinguished from the latter by the characteristic feature of the axial filament.

The degree of development of the axial filament varies in different species. In some it is thick and distinct, while in others it is quite slender, indistinct, and even almost unnoticeable in some species. In some forms it is almost uniform in thickness throughout its entire length; in others it gradually diminishes in thickness towards the posterior end, while yet in others it is indistinct in only the middle portion. The filament does not hang freely in the endoplasm, but its posterior end is fixed at the posterior extremity of the body. The axial filament is, moreover, usually adherent to the body wall for the greater part of its length, and it is not capable—as in *Pyrsonympha*—of independent lashing movements in the cytoplasm. In two species (*D. leidyi*

and *D. parva*) it is very indistinct and hardly visible in the majority of individuals, except at the anterior end—probably owing to its slenderness.

Just as in *Pyrsonympha*, the anterior tip of the axial filament is situated at the anterior tip of the body, and the flagellar cords arise at this point. In well-developed axial filaments, the anterior end is often slightly thickened, and two parts are then distinguishable at this end; namely, a main central portion, which is faintly stained, and a peripheral zone which stains intensely.

The mode of arrangement of the flagellar cords on the surface of the body differs from that in *Pyrsonympha*. The cords run parallel with the longitudinal axis of the body, and their distribution is not uniform over the entire surface, but is limited to one-half of the surface or a little more. It is on the surface occupying the outside in the spirally curled body that the flagellar cords are found, the surface occupying the inside being destitute of them. The flagellar cords are straight in their course, those of only one species, viz. *D. rugosa*, forming an exception, where they are fairly wavy. In the majority of the species, each cord is situated on a distinct ridge, so that the outline of the organism appears beautifully serrated; but in a few species the ridges are absent or very indistinct, and the surface appears entirely or almost smooth. The surface destitute of cords is smooth in all species. The free portions of the flagellar cords at the posterior end of the body commonly measure from one-fifth to one-fourth of the body length, except in one species (*D. leidy*) where they are conspicuously long.

In some species the body is entirely naked, while in many others peculiar filamentous appendages, such as are seen in *Holomastigotoides* and *Pyrsonympha*, are found on it. In some forms these appendages are present in large numbers, and are distributed over the greater portion of the surface of the body, while in others they are limited to a small area. Their mode of distribution on the body is rather regular, and in one form they are arranged in very regular transverse rings. The nature of these filamentous structures will be considered afterwards.

The nucleus is situated at the anterior end, except in one species (*D. rugosa*), in which it is found in the middle portion of the body. In one other species (*D. exilis*) individuals with a terminal nucleus and those with it separated from the anterior end are both common. I am not sure whether or not such individuals are ever to be seen in the other species. The internal structure of the nucleus is of the same type as that of *Pyrsonympha*. When situated at the anterior end of the body, it is oval or piriform. In the majority of forms its posterior end is rounded, whilst it narrows towards the anterior pole. The narrowed end of the nucleus is usually found at the extreme anterior end of the body, where the anterior tip of the axial filament is also fixed. From this disposition, it cannot be doubted that the nucleus and the axial filament are kept in connexion with each other, just as in the forms of *Pyrsonympha*. The nucleus when localized at the middle region appears commonly round, but one peaked point is often distinctly noticeable (Pl. XV, fig. 72) which may be taken

as an indication of the existence of a connexion between the nucleus and some other part of the body—perhaps the axial filament, as in the other forms. As mentioned already, the American authors did not describe any connexion between the nucleus and the axial filament in the *Pyrsonympha* studied by them; but the shape and the position of the nucleus, as illustrated by them, show resemblances to the conditions in some of the species of *Dinenympha* studied by myself, and I am of the opinion that a special structure connecting the nucleus and the axial filament exists also in the American species of *Pyrsonympha*.

Like *Pyrsonympha*, the species of *Dinenympha* live both hanging from the intestinal wall and swimming freely in the lumen of the gut. Freely swimming individuals move very vigorously. They display both rotation and flexion, and in slender forms the anterior end is actively swung in all directions.

Dividing forms are met with very rarely. The process of division seems, as far as I could conjecture from several stages that came under my notice, to be of the same type as that observed in *Pyrsonympha* (Pl. XV, figs. 83 and 84).

Several forms are to be distinguished in this subgenus, and in my earlier account (1917) I described them as nine species and one variety. At present, however, I feel that it is better to regard the independence of some of the forms as questionable, and here I propose to classify them under six species and one doubtful species.

The forms harboured by *Leucotermes speratus* of Japan proper and those in *Leucotermes flaviceps* of Formosa are not identical, three species and the doubtful one being common to both species of termite.

(1) *Dinenympha exilis* sp. nov. (Plate XV, figs. 67 and 68,
and Text-fig. E, a).

The body is long and slender, the greater portion being almost uniform in thickness though it tapers gradually at the extremities. It measures 50–100 μ in length and 4–8 μ in width at the middle. The number of complete spiral turns of the whole body is commonly two or two and a half: so that in side view four or five undulations are commonly visible. When the organism does not move very actively, its body is oval or elliptical in cross section; but when it moves actively the body becomes somewhat more flattened. The surface of the body is almost smooth, the ridges on which the flagellar cords are situated being very indistinct. The axial filament is thick, but becomes slightly thinner towards the posterior end. The endoplasm is clear and food débris is rarely contained in it. The nucleus is of an elongated oval shape, and is situated at the anterior extremity of the body, as a rule; but occasionally it is found near the middle or in the hinder regions.

This species is found in both *Leucotermes speratus* and *L. flaviceps*. No forms taken to be of this type are found in the descriptions of the American authors.

- (2) *Dinenympha rugosa* sp. nov. (Plate XV, figs. 69–72, and Text-fig. E, b).

The body is long and slender as in the preceding species; but it is flat and ribbon-like, both sides being rather distinctly edged, and it tapers more abruptly at the posterior end. The length is almost equal to that of *D. exilis* but the width is about twice as great. The surface is not smooth but is provided with high and distinct ridges, and the flagellar cords are markedly wavy. In young individuals with only four cords the ridges are particularly prominent. The axial filament is slender in this species, in contrast with *D. exilis*, being slightly thicker than the flagellar cords at the anterior portion and nearly equal to them at the hinder end, so that it is here hardly distinguishable from them. The endoplasm appears remarkably coarse and is frequently vacuolated, showing an appearance quite different from that of *D. exilis*. The nucleus is situated near the middle of the body. It is commonly round in shape, but in some forms a peaked point is noticed, as shown in Pl. XV, fig. 72, which may be taken as an indication of the existence of a connexion between the nucleus and a certain portion of the body.

The species is harboured by *Leucotermes speratus* only. Forms of this type appear to be lacking in the descriptions of American authors.

- (3) *Dinenympha nobilis* sp. nov. (Plate XV, figs. 73–75, and Text-fig. E, c).

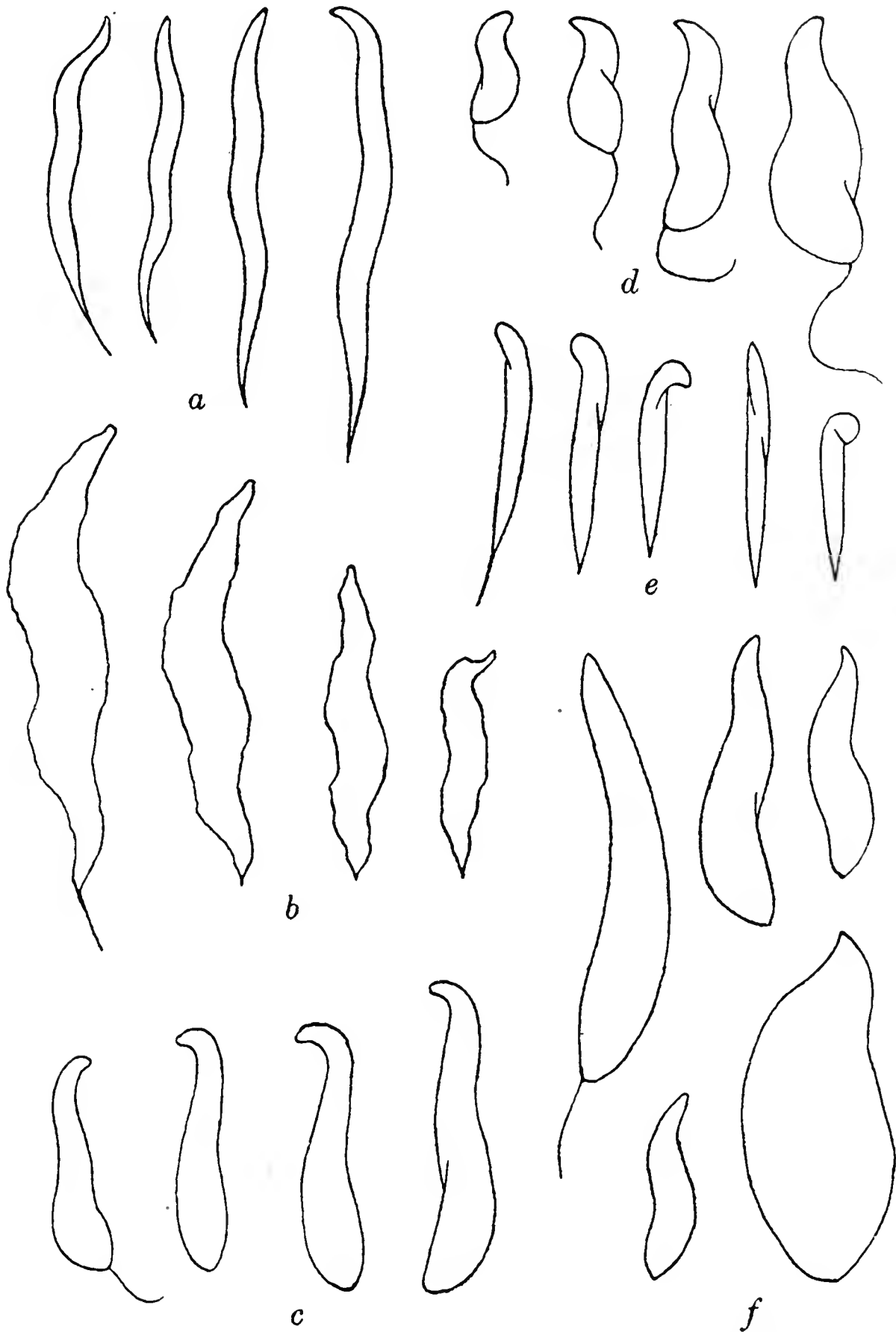
The body assumes a club shape, gradually thickened towards the posterior end, and measures 30–60 μ in length and 5–10 μ in width. It is almost evenly rounded at both ends and is oval in cross section, the surface being quite smooth. Twisting of the body is slight, and the anterior portion is usually turned back, so that it assumes commonly the shape of a hook. The axial filament is rather indistinct. It is fairly thick anteriorly and becomes gradually slender towards the middle, where it is usually almost invisible; but it then becomes gradually thicker again posteriorly, and the extreme end is specially thickened. The endoplasm is of a peculiar appearance in this species: it is dark and finely granular during life, usually containing plenty of food débris.

This species seems lacking in the Formosan species of termite, having been found in *Leucotermes speratus* only. No forms of this type seem to have been illustrated by the American authors.

- (4) *Dinenympha leidy* sp. nov. (Plate XV, figs. 76 and 77, and Text-fig. E, d).

The body is lanceolate and is fairly thick at the posterior portion. The ridges on the surface are very distinct and are arranged regularly. The axial filament is very indistinct, being made out with difficulty at the anterior region, and invisible at the posterior. The most remarkable feature of this form is that the free ends of the flagellar cords are conspicuously long, reaching

a length equal to about one-half that of the body. Filamentous appendages are found usually distributed thickly over a limited area of the surface, free from the flagellar cords, at the posterior end of the body, and small numbers of them are also found on the other parts of the body. The length of the



Text-fig. E. Outline drawings of the species of *Dinemypha*. Drawn with camera lucida. ($2 \times \frac{1}{12}$.)
a, *D. exilis*; *b*, *D. rugosa*; *c*, *D. nobilis*; *d*, *D. leidy*; *e*, *D. parva*; *f*, *D. porteri*.

organism is usually between 25μ and 50μ , the width some 8μ to 15μ . The nucleus is spherical or oval, and situated at the anterior extremity.

This form is found in common in both the Japanese species of *Leucotermes*. The forms described by Leidy under the name of *Dinemypha gracilis* appear

to be similar, but this latter species is distinguishable by its greater length and more tapering posterior end; and apparently its free flagella are shorter, judging from the figures of Leidy and Porter.

(5) *Dinenympha parva* sp. nov. (Plate XV, fig. 78, and Text-fig. E, e).

This is the smallest member of the whole group, measuring $20\text{--}45\mu$ in length and $3\text{--}5\mu$ in width. The body is slender and appears almost straight, excepting the anterior portion, which is often curved or bent back. It appears simply twisted, but it is also wound slightly in a spiral in many individuals. The ridges on which the flagellar cords are situated are very indistinct, and the surface of the body is almost smooth. The nucleus is spherical and is situated at the anterior extremity of the body. The axial filament is very slender and indistinct, being made out only in exceptional cases, and is found at the anterior end only. The endoplasm has a characteristic appearance, containing commonly a large quantity of globular bodies taking iron-haematoxylin rather deeply. The peculiar filamentous appendages are found in small numbers on the posterior portion, and sometimes also at the anterior end.

This species occurs in *Leucotermes speratus* and *L. flaviceps*. No forms closely resembling it have ever been described by previous authors.

(6) *Dinenympha porteri* sp. nov. (?) (Plate XV, figs. 79–82, and Text-fig. E, f).

The body assumes a fusiform or lanceolate shape, pointed at both ends or rather rounded at the posterior end. It measures $25\text{--}80\mu$ in length and $6\text{--}15\mu$ in width. In some forms the body is somewhat flat and its edges are rather distinct, while in others the body is fairly thick and no distinct edges are recognizable. The axial filament is thick and distinct throughout its entire length, and a rhombic enlargement of it can be recognized at the posterior end in some specimens (Pl. XV, fig. 82). Some individuals are entirely naked, as in the preceding species, while in others the filamentous appendages are found only on certain portions, or over almost the entire surface of the body. As regards the coat of filamentous appendages, and the other characters, the following four types are distinguishable.

Type 1. The body is lanceolate and edged at both sides, $30\text{--}60\mu$ in length and $7\text{--}15\mu$ in width. No filamentous appendages are present (Pl. XV, fig. 79).

Type 2. The shape and size of the body as in the forms of the preceding type. A group of filamentous appendages, varying from about 12 to 24 in number, is found at the posterior end of the body. At the anterior end, moreover, one or two pairs of filamentous appendages are found. These filamentous structures are commonly longer and thicker than those at the posterior end of the body: they differ in appearance from the latter, and in some individuals their attachment to the anterior end of the axial filament can be made out (Pl. XV, fig. 80).

Type 3. The body is lanceolate or club-shaped, and its edges may be distinct or indistinct. Commonly the organism is longer and more slender than those of the above two types, measuring 25–80 μ in length and 6–10 μ in width. The axial filament is very thick. The body is thickly covered with filamentous appendages, diffusely distributed over almost the entire surface. At the posterior region they are particularly dense, and at the anterior end they are somewhat denser than in the middle portion (Pl. XV, fig. 81).

Type 4. Resembles the forms of the foregoing type, and is usually indistinguishable in fixed and stained preparations. The mode of arrangement of the filamentous appendages, however, is very peculiar in this type. They are not distributed diffusely on the surface but are arranged in transverse rows varying from 12 to 18 in number. The body is a little smaller than in the preceding type.

Whether any or all of these types should be regarded as distinct species or not is a question hard to answer decidedly at present. To give a correct answer the nature of the filamentous appendages must be exactly determined. My observations in this respect being still inadequate, my knowledge of these structures is limited. Some of the filaments, such as those seen at the posterior end of the body of the forms of Type 2, are probably micro-organisms attached to the body wall. Some of them, however, such as those at the anterior end of the forms of the same type, seem to have certain peculiarities, if taken for attached micro-organisms. As for those such as are seen in Type 4, I am of the opinion that they should not be regarded as micro-organisms, but are rather similar to, or homologous with, the "bristles" described by Janicki (1915) in *Parajoenia*. In my former paper in Japanese I described each of these types under a specific name (*D. nuda*, *D. corniculata*, *D. porteri*, and *D. comosa* respectively); but now I think it better to leave the question of their identity in doubt, provisionally calling the group by the name *D. porteri*.

PART II. GENERAL DISCUSSION.

1. COMPARATIVE MORPHOLOGY.

The forms belonging to the *Trichonympha* series are of very varied organization, and the differences among them are so striking in many cases that one cannot help doubting whether they belong to one and the same group in the system. Both the forms of complicated organization, as well as those of rather simpler structure, are provided with some peculiarities. The members of the *Pyrsonympha* series also have many peculiarities in their organization. Consequently, the comparative morphological study of all these forms is most interesting and important. Notwithstanding, however, a rather rapid advance in our knowledge in recent years, this subject has hardly been touched upon by any authors: but as I have fortunately been able to study many forms and, especially, the process of division in several of them, I shall undertake to discuss these questions here.

Let us begin with the comparative study of the most complicated and most peculiar organs in *Trichonympha*, *Teratonympha*, and *Pseudotriconympha*; namely, the structures in the nipple and those in connexion with the nucleus. It will be helpful to give here a brief review of the structures which I am going to consider. In *Trichonympha*, there is an *axial core* consisting of two parts, viz. the wall and its contents; the nucleus is held at the bottom of a *corbule*; and there is also a *columnar body* hanging perpendicularly from the base of the axial core to the anterior pole of the nucleus. In *Teratonympha*, the *axial core* is a solid cone, but is surrounded by a special layer of protoplasm, filling up the space between it and the *tubular rind*; the nucleus is enveloped in a peculiar *nuclear sac*, which is closely connected with the body wall and the above-mentioned layer by means of its flange and its neck respectively. I conjecture that the protoplasm around the core is of the same substance as that of the nuclear sac. I conjecture also that the contents of the axial core of *Trichonympha* is homologous with the layer around the axial core in *Teratonympha*, and that the nuclear sac of *Teratonympha* is also homologous with the corbule and the columnar body in *Trichonympha*. The contents of the axial core of *Trichonympha* is granular, but the layer around the axial core of *Teratonympha* is homogeneous. Thus it may seem unreasonable to take the two to be of the same substance; but I think the contents of the axial core of *Trichonympha* consists really of two substances, viz. a homogeneous ground substance and granules embedded in it—the former being comparable with the protoplasm around the axial core in *Teratonympha*, while the granules are lacking in the latter. Thus I interpret these two genera to show a rather distinct similarity in organization, as regards the points discussed above; the differences being the relative position of the two components in the nipple, and the greater differentiation of the axial core in *Teratonympha*.

Resemblances between *Trichonympha* and *Pseudotriconympha* are distinct and easily recognizable. The *axial core* of *Pseudotriconympha* is clearly comparable with that of *Trichonympha*. The structures in *Pseudotriconympha*, consisting of a *tubule* and a *ball*, may be safely taken to represent a type of structure quite similar to that of *Teratonympha*, and they may also be taken to show a further differentiation of the axial core, with its column and knob-like end, of *Trichonympha*. A difference, however, is recognizable between *Trichonympha* and *Pseudotriconympha*: for neither a corbule, nor any structure corresponding to it, is distinguishable in *Pseudotriconympha*.

As regards the rôle played by the axial core of *Trichonympha* during the process of division, the observations of the Italian and American authors agree in essential points with my own. In this genus, the mitotic figures are not formed from the nucleus only, but the strand ("fuso esterno" of Foà, "paradesmose" of Kofoed and Swezy) and the division-centre are of extranuclear origin, being furnished by the nipple. Foà, and Kofoed and Swezy, agree in the view that the axial core plays the part of a division-

centre in mitosis. I also failed to find any structures such as centrosomes at the ends of the strand, and I agree with the above view. As for the origin of the strand, I think it is probably derived from the wall of the axial core.

In *Teratonympha* and *Pseudotrichonympha* the condition is somewhat different. Here the axial core does not function as a division-centre, but a special structure is formed for the purpose. In the early stages of division of *Teratonympha* a strand is seen, quite independent from the nucleus, provided with a "central body" at both its ends, each of these being connected with the base of the daughter nipples by means of a thread. As regards the origin of these structures, I think they are derived from the axial core. Thus I believe that the axial core of both *Teratonympha* and *Trichonympha* is the source of the structures of extranuclear origin in mitosis; but these organisms differ in one point, namely, in *Teratonympha* the axial core gives rise to the body functioning as a centrosome, whilst in *Trichonympha* it acts itself as a division-centre. In *Pseudotrichonympha* the condition is quite similar to that of *Teratonympha*: namely, the spherical body and the thread connecting it with the nipple are derived from the tubular column, and probably, I think, from its wall, whilst the strand arises from the spherical body.

The homology of the two layers at the anterior region of *Trichonympha*, *Teratonympha*, and *Pseudotrichonympha* is readily recognizable; the differences being simply in degree of development. The axial core in *Trichonympha* is surrounded concentrically by the inner and outer layers, the basal granules of the flagella being localized at the basal or proximal surface of the outer layer. In *Pseudotrichonympha* almost the entire surface of the axial core is directly surrounded by the outer layer, and only a small portion of its posterior part is in contact with the inner layer; consequently the basal granules, in this region, are localized immediately on the surface of the axial core. In the bell, the distribution of the layers is identical in both genera. In *Teratonympha* the basal granules of the flagella are found at the base of the outer layer, just as in the other genera, and the conical axial core is in contact with the inner layer (tubular rind) at its anterior end. As they are partly separated by the layer of peculiarly differentiated protoplasm, the axial core and the inner layer of *Teratonympha* seem not so intimately connected as in the other genera. But it is not only at the anterior end that these two parts are connected: there is also the vertical partition crossing the space between them, and keeping them in close connexion. Thus the inner layer seems to serve as a means of connexion between the locomotory organs and the part of the body which plays the rôle of the division-centre in mitosis.

In *Microspironympha* the anterior portion of the body is not so highly differentiated as in the above three genera; but there is a peculiar tubular structure situated in the axis of the body, connecting the tip of the body and the anterior surface of the nucleus. It is easily imaginable that this is a structure corresponding to the axial core in *Trichonympha*. Moreover, it seems not unreasonable to interpret the tubular wall and its contents as

respectively homologous with the wall of the axial core and its contents in *Trichonympha*. My knowledge of the process of division in this species is incomplete, but it seems highly probable that the slender strand and the structures acting as division-centres at both its ends are derived from the tubule. Each row of basal granules starts at the tip of the tubule, and the relation between them seems also analogous with what is seen in *Trichonympha*. There is, moreover, a structure which I conjecture to be homologous with a nuclear sac or corbule. This is the special protoplasm enveloping the tubule and the nucleus. The outer boundary of this layer of protoplasm is not distinctly contoured, but it is especially distinct at the base of the tubule and the anterior pole of the nucleus. As described in a previous section, the posterior end of the tubule is commonly found fixed on the nuclear wall; but sometimes they are separated from each other and connected by means of a mass of dense protoplasm, in which the contents of the tubule and the special protoplasm around it are not distinguishable. Thus I think there are many reasons indicating that the dense protoplasm and the contents of the tubule are composed of the same substance; and, moreover, that they are homologous with the nuclear sac, the corbule, and the contents of the axial core. From the above data, it may be concluded that the structure of *Microspironympha* is similar to that of *Trichonympha*, but of a less complex type.

In *Holomastigotoides*, the organization of the anterior portion is apparently of a quite different type. There is no structure corresponding to the tubule in *Microspironympha* or the axial core in *Trichonympha*. All parts of the mitotic figure seem to be formed from the nucleus itself, and both the division-centre and the strand appear on the nucleus: so that it is reasonable to suppose that a body which acts as a division-centre is localized on the nuclear wall. The most interesting structure of *Holomastigotoides*, from the viewpoint of comparative morphology, is the peculiar mass of special protoplasm surrounding the nucleus and occupying the area in front of it. It is clearly observable in good preparations that the anterior tip of this mass reaches the anterior extremity of the body, where the ends of the rows of basal granules of the flagella are kept in connexion with it. That is to say, in this genus the body playing the rôle of the division-centre lies separated from the basal granules of the flagella, but they are kept in connexion by means of the above-mentioned protoplasm. I believe that it is reasonable to interpret this structure as homologous with the dense protoplasm and the contents of the tubule of *Microspironympha*, and consequently with the corbule of *Trichonympha* and the nuclear sac of *Teratonympha*.

In some individuals of *Holomastigotoides* the particular protoplasm is remarkably developed, assuming the form of a distinct and fairly long column hanging along the longitudinal axis of the body. In *Microspironympha* some individuals are found provided with a structure at the posterior portion of the body, appearing sometimes as a well-developed transparent style. I am of the opinion that both these structures are similar in nature,

and that the style in *Microspironympha* is of the same substance as, and most probably continuous with, the contents of the tubule and the layer surrounding the nucleus. Furthermore, I conjecture that these structures in *Microspironympha* and *Holomastigotoides*, together with the nuclear sac in *Teratonympha* and the corbule in *Trichonympha*, are homologous with the axostyle and the structure enveloping the nucleus in *Joenia*, *Devescovina*, *Lophomonas*, and related forms.

As for the other two genera of the *Trichonympha* series which I have studied, viz. *Spirotrichonympha* and *Holomastigotes*, no dividing stages were observed, and our knowledge of these forms is too scanty for discussion. But I believe that these forms represent types similar to, but simpler than, that of *Holomastigotoides*. There is a mass of dense protoplasm connecting the anterior ends of the rows of basal granules with the nucleus; and I suppose that the latter gives rise to all parts of the mitotic figure.

Let us now consider the structure of the members of the *Pyrsonympha* series. The organization of these forms appears quite simple, but it is, in reality, not less peculiar than that of the *Trichonympha* series. I distinguished many species in this group, and have classified them in two subgenera of a single genus, viz. *Pyrsonympha* and *Dinenympha*. All of these species are undoubtedly of the same type in essential points of organization, showing differences only in degree of development or differentiation of some organs. To make this clear, a brief recapitulation of the structure in *Pyrsonympha grandis*—the species showing the characteristic organization most clearly—may be given here. First, there is an *axial filament*, running in the long axis of the organism, and ending anteriorly at the tip of the body; secondly, there are the *flagellar cords* which arise at the anterior tip of this filament and run backwards, attached on the body wall, to the posterior end of the organism; lastly, there is the nucleus, enveloped in the *nuclear sac*, which is kept in connexion with the axial filament and the body wall. Towards the anterior end, the outer wall of the axial filament stains more deeply than the internal part with iron-haematoxylin, although the boundary between the two parts is not distinct and no definite zones are visible. At the anterior extremity, a sharp differentiation into these two constituents is frequently observable. In some individuals, however, a small portion of the anterior end appears to consist of an axis of feebly stainable substance with deeply stainable cords of almost uniform thickness regularly arranged along its surface. Thus it seems certain that the axial filament consists of two components, which are distinctly differentiated only at the anterior end. In small individuals of *Pyrsonympha grandis*, the axial filament is commonly homogeneous throughout its entire length. In the other species of smaller size, and in the species of *Dinenympha*, the axial filament also commonly appears homogeneous throughout its entire length. In some individuals, however, differentiation into an outer wall, staining intensely with iron-haematoxylin, and a feebly staining central part, may be observed on rare occasions. The most important feature in the

organization of *Pyrsonympha* is the apparent absence of any basal granules for the flagellar cords. From the stainability of the axial filament and its mode of connexion with the flagellar cords, I think it not unreasonable to conjecture that this organ itself functions as a basal granule or blepharoplast for the flagellar cords, and I would regard the component taking iron-haematoxylin deeply as specially exercising this function.

In my opinion the axostyle of trichomonad flagellates is not homologous with the axial filament of *Pyrsonympha*. The axial filament is not simply a skeletal organ like the axostyle, but an organ playing the rôles of several different structures in the trichomonads, viz. the axostyle and the basal granules.

As the nucleus of *Pyrsonympha* divides, the axial filament becomes detached from it, and the centrosomes make their appearance on the nuclear wall, with half the number of flagellar cords starting from each of them. If my interpretation given above—attributing the nature of a basal granule to the axial filament—be accepted, it may be deductively conjectured that the filament supplies the bodies which play the part of division-centres. In this respect, the axial filament may be taken to be homologous with the axial core in *Trichonympha* and the corresponding structures in other forms of the *Trichonympha* series. The nuclear sac in *Pyrsonympha* seems homologous with the nuclear sac in *Teratonympha* and the corbule in *Trichonympha*, on account of its relations to the axial filament, the body wall, and the nucleus. I am inclined, moreover, to suppose that the part of the filament which does not stain with haematoxylin consists of the same material as the contents of the nuclear sac.

2. CLASSIFICATION.

A great many types are represented by the protozoal organisms harboured by termites. Not only do they differ from each other, but the great majority of them display so many peculiarities that a large number of genera had necessarily to be established for them. At present 38 generic names are recorded in the literature (excluding spirochaetes and gregarines), and I have added two more in this paper. The greater part of this large number of genera, namely 36, consists of those established for organisms found in the intestines of termites exclusively. Among these 36 names, at least three are to be taken as synonyms of others; and in a few of the remainder the descriptions are not sufficient to enable us to understand their characteristics distinctly. A synopsis of the genera recorded from termites has already been given on p. 243.

The organisms peculiar to the termites may be divided into two distinct groups; namely, those provided with numerous or abundant flagella, and those with a small number of these structures. Our "*Trichonympha* series" comprises several types of the former group, while the "*Pyrsonympha* series" contains several organisms representing only a single type of the latter.

The first serious attempts to classify these organisms, and to arrange them in a system, were made by Grassi (Grassi, 1892; Grassi and Sandias, 1893; Grassi [and Foà], 1911). In 1911 he established a new order, Hypermastigina, in the Flagellata, for the group of organisms provided with numerous flagella, and suggested that three families might be distinguished. These families were not named, however. The first would include *Eulophomonas*, *Lophomonas*, *Mesojoenia*, *Joenia*; the second *Trichonympha*; the third *Spirotrichonympha*, *Holomastigotes*, and *Holomastigotoides*. But Grassi remarks that *Microjoenia* and *Pseudotrichonympha*—which do not fit into this system—make it difficult to arrange the organisms in question in groups higher than genera. The forms with a small number of flagella he classified among the Polymastigina, and established two new families, viz. Calonymphidae and Dinenymphidae, for them.

After Grassi, França (in a paper sent to the editor in 1914 but not published till 1916) and Janicki (1915) published their views on the classification. França divided the Hypermastigina into four families, giving family rank to the genus *Lophomonas*; his four families being Calonymphidae, Lophomonadidae, Trichonymphidae, and Holomastigotidae. Janicki proposed, following Grassi, to classify the Calonymphidae among the Polymastigina, and to divide the Hypermastigina into the following four families: Lophomonadidae, Joenidae, Trichonymphidae, and Holomastigotidae. In his most recent paper, Grassi (1917) has described a large number of forms which he classifies in eight families, belonging to two different orders. In the Hypermastigina he distinguishes five families, viz. Joenidae, Staurojoenidae, Trichonymphidae, Spirotrichonymphidae, and Holomastigotidae. The rest of the forms, which he assigns to the Polymastigina, are classified in the three families Tetramitidae, Dinenymphidae, and Calonymphidae.

As many of the organisms are still not thoroughly investigated, their classification can but be, at present, provisional, and alterations and improvements will inevitably accompany progress in our knowledge. The forms studied by myself belong to more than a few types, but not to so many as to enable me to attempt a final classification of the entire group of these organisms. Accordingly, it is rather my intention in this section to consider the classification of those forms only which I have myself studied, and to make clear the characters of the genera and families to which these forms belong.

Trichonympha and *Pseudotrichonympha* are closely similar, and it is reasonable to place them near one another in the system. *Teratonympha*, as remarked in the preceding section, shows a rather close resemblance, in the structure of the anterior portion of its body, to *Trichonympha* and *Pseudotrichonympha*. *Teratonympha*, however, displays some distinct peculiarities in both the anterior and posterior regions of its body; and I think these sufficient to justify me in establishing a new family, which I propose to call Teratonymphidae, for these organisms.

In the system of Grassi, *Holomastigotes* and *Holomastigotoides* are classified separately from *Spirotrichonympha*. The differences between these genera, however, do not seem to me so remarkable as to warrant their separation into different families. The peculiarly differentiated protoplasm in the anterior region of the body is similar in the three genera, differing simply in the degree of its development. The organisms agree also in the character that the flagella are arranged in spiral rows: and in all these forms, also, the spiral arrangement is peculiar in being dextrotropic. *Spirotrichonympha* seems to differ from the other genera in that the basal granules of the flagella lie somewhat deep in the endoplasm; but I do not think this difference to be very important. As for my *Microspironympha*, it has the rows of flagella running spirally (dextrotropic), just as in the above three genera; but it differs from them in being provided with the tubular structure connecting the anterior tip of the body with the nucleus. The differences between the Holomastigotidae and Spirotrichonymphidae of Grassi are not so distinct as the differences between *Microspironympha* and any of the Spirotrichonymphidae and Holomastigotidae. Consequently if *Holomastigotes* and *Spirotrichonympha* are taken to belong to different families, *Microspironympha* would naturally have to be separated from both of them. But I do not think the differences among *Microspironympha*, *Holomastigotes*, and *Spirotrichonympha* are so important that each must be taken to represent the type of a different family. I am of the opinion that these genera should all be put at present in a single family, which may be called Holomastigotidae.

The forms provided with a smaller number of flagella are classified among the Polymastigina by Grassi. They are of very varied types and the great majority of them have their own peculiarities: so I suppose that, as our knowledge increases it will ultimately be necessary to establish many families for them. For instance, some of the forms classified by Grassi among the Tetramitidae will perhaps soon be separated from the ordinary trichomonads. In this group, my material was limited to only one genus, *Pyrsonympha*. *Pyrsonympha* has very distinct characteristics, and it is unquestionable that it has to be separated from any families established for flagellates other than those from the intestines of termites. I cannot, indeed, find any form among hitherto described flagellates which seems to belong to the same family: for I cannot agree with Grassi (1917) in ranking *Pseudotrypanosoma*, described by him, in the same family with *Pyrsonympha*.

The families and the genera studied by myself will be defined as follows:

(1) Family *Trichonymphidae* S. Kent (emend.).

Large forms. Anterior part of the body highly differentiated: body wall of the anterior portion formed of one or two thick ectoplasmic layers, densely traversed by numerous flagella: anterior extremity provided with an axial skeleton (axial core). A single large vesicular nucleus present. There is no

permanent mouth, food being ingested through the general surface of the hind end of the body. A peculiar differentiation of the protoplasm surrounding the nucleus is present in the anterior region. It may assume the form of a hemispherical bowl (corbule) and a columnar mass; or it may be distributed diffusely, or merely form a layer round the nucleus. The rest of the body not distinctly ridged; naked or flagellated. Flagella arranged in longitudinal rows at anterior end of the body, and sometimes restricted to this region. Sometimes, however, the rows extend over the greater part of the body, and in this case they are arranged in spiral lines (laeotropic) in the posterior region. Multiplication by simple longitudinal fission, the nucleus dividing by mitosis. No cysts or sexual stages known.

Genus I. *Trichonympha* Leidy, 1877.

Anterior part of the body distinguishable into two parts, the nipple and the bell. The nipple consists of an axial core with two layers surrounding it. The wall of the bell also consists of two similar layers. A corbule and a columnar mass of peculiar protoplasm are present, the nucleus being kept at the bottom of the former and at the base of the latter. Flagella arising in longitudinal rows from nipple and bell. The hinder part of the body, posterior to the bell, free from flagella¹. Type species *T. agilis* Leidy, 1877. (In *Leucotermes* (*Reticulitermes*) *flavipes*, N. America.)

Genus II. *Pseudotrichonympha* Grassi, 1911.

Anterior part of the body distinguishable into two parts, as in *Trichonympha*. The axial core consists of a column with a ball at the tip, and is surrounded by a single layer of ectoplasm. The wall of the bell consists of two layers. The nucleus lies freely in the endoplasm. Body posterior to bell furnished with spiral rows (laeotropic) of short flagella, excepting a small area at the posterior extremity. Type species *Ps. hertwigi* (Hartmann) Grassi, 1911. (In *Coptotermes hartmanni*, Brazil.)

(2) Family *Teratonymphidae* (fam. nov.).

Large forms. Anterior end of the body highly differentiated as in the *Trichonymphidae*, but more complex in structure. Body regularly ridged transversely, so that it appears metamerically segmented. Each ridge provided with a single row of flagella. No visible mouth. The single vesicular nucleus is enclosed in a nuclear sac, fixed to the body wall and base of the head, and lies in the first apparent segment of the body. Multiplication by simple longitudinal division, with mitosis of nucleus. No cysts or sexual development known. Genus *Teratonympha* (gen. nov.) with characters of family. Type species *T. mirabilis* mihi. (In *Leucotermes* (*Reticulitermes*) *speratus*, Japan.)

¹ If the description of Kofoid and Swezy (1919) is right, I do not take their *Trichonympha campanula* to belong to this genus. *Leidyopsis* described by them appears to be closely similar to (probably synonymous with) *Gymnonympha* of Dobell, and I think both belong to this family.

(3) Family *Holomastigotidae* Grassi (emend.).

Body not distinctly divided into different parts—as in *Trichonympha*. Flagella arranged in spiral rows from their commencement at anterior end; direction of spiral dextrotropic. A part of the hind end of the body—which may be large or small—free from flagella. A peculiar mass of dense protoplasm exists at the anterior portion of the body, and surrounds the nucleus, but in some genera this mass is less dense in the immediate vicinity of the nucleus. A tubular organ in connexion with this structure may or may not be present. Surface of the body ridged or apparently smooth. Nucleus single, vesicular. No visible mouth. Multiplication by simple longitudinal division, with mitosis of nucleus. No cysts or sexual stages known.

Genus I. *Holomastigotes* Grassi, 1892.

Small forms. Surface of the body with spiral ridges, few in number. Basal granules of flagella situated at the bottom of the groove behind each ridge, the basal parts of the flagella being fixed on the surface of the ridges. A dense mass of protoplasm is present at the anterior end, the nucleus being embedded in it. Nucleus anterior, spherical. Type species *H. elongatum* Grassi, 1892. (In *Leucotermes* (*Reticulitermes*) *lucifugus*, Italy.)

Genus II. *Holomastigotoides* Grassi, 1911.

Large forms. Surface of the body with numerous spiral ridges, the flagella being attached as in the preceding genus. A mass of dense protoplasm is present at the anterior portion, and distinctly developed: it surrounds and sometimes extends behind the nucleus. Nucleus anterior, ovoid or compressed antero-posteriorly. Type species *H. hertwigi* (Hartmann) Grassi, 1911. (In *Coptotermes hartmanni*, Brazil.)

Genus III. *Spirotrichonympha* Grassi, 1911.

Medium-sized forms. The rows of basal granules of the flagella lie somewhat more deeply in the endoplasm than in the preceding genera; being deepest at the anterior end and becoming more superficial towards the posterior. A fairly large part of the root of each flagellum is thus embedded in the protoplasm. The mass of dense protoplasm assumes the shape of a cone, its hinder border being indistinct. The spherical nucleus lies apparently free in the endoplasm, at some distance from the anterior extremity. Type species *Sp. flagellata* Grassi, 1892. (In *Leucotermes* (*Reticulitermes*) *lucifugus*, Italy.)

Genus IV. *Microspironympha* (gen. nov.).

Small forms. Surface of the body not ridged: the rows of basal granules lie under the surface, but are somewhat more deeply placed at the anterior end. Nucleus spherical, anterior, but separated from the anterior extremity

by a tubular structure, connecting its anterior pole with the anterior tip of the body. The spiral rows of flagella arise from the anterior end of this structure. A mass of denser protoplasm is also present surrounding the tubule and the nucleus. An axial rod sometimes (? always) present. Type species *M. porteri mihi*. (In *Leucotermes* (*Reticulitermes*) *speratus*, Japan.)

The three foregoing families belong to Grassi's order Hypermastigina. The remaining family contains organisms which differ considerably from all those just considered.

(4) Family *Pyrsonymphidae* Grassi.

Large or small forms. An elastic thread (the axial filament), of variable thickness, runs longitudinally down the body. From the anterior tip of the body a small number of flagellar cords start at the anterior extremity of this filament, and run spirally backwards attached on the surface of the body, becoming free as flagella at the posterior end. Spiral direction of cords laeotropic in all species studied. Nucleus single; variable in shape but usually anterior or central in position.

Genus *Pyrsonympha* Leidy, 1877 (*emend.*).

Body piriform, club-shaped, spindle-shaped, or screw-like: simply twisted, or both twisted and spirally wound. Number of flagellar cords 4 or 8.

Subgenus I. *Pyrsonympha* Leidy (*emend.*).

Body piriform, club-shaped, or screw-like; spirally twisted. The axial filament hangs in the endoplasm, its posterior end being free from the body wall. Type *P. vertens* Leidy, 1877. (In *Leucotermes* (*Reticulitermes*) *flavipes*, N. America.)

Subgenus II. *Dinenympha* Leidy (*emend.*).

Body slender and wound spirally, sometimes also spirally twisted. Axial filament ends fixed at the posterior tip of the body, or is indistinct except at the anterior end. Type *D. gracilis* Leidy, 1877. (In *Leucotermes* (*Reticulitermes*) *flavipes*, N. America.)

As regards the systematic position of the *Pyrsonymphidae* I am still in doubt. Most recent authors—including Grassi—place this family among the Polymastigina: but the organisms appear to me so different in many ways from all the other forms in this order, that I hesitate to assign them to this group. It seems to me that it will be necessary to found a new order to contain them; but as the classification of the whole order Polymastigina is still defective, owing to our incomplete knowledge of many forms, I shall not propose a name for this suggested new order at present.

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DESCRIPTION OF PLATES X—XV.

All the figures, except diagrammatic ones (Figs. 13, 15, 35, 53, 75, 82) were drawn with a camera lucida. The optical apparatus employed was as follows: Zeiss stand IA, immersion objective $\frac{1}{12}$ ", and Huygenian oculars 2 or 4; tube of microscope not drawn out, and fitted with revolving nosepiece. Drawings made at level of stage of microscope. The dimensions of the organisms are stated in the text. In some figures the surface of the organism is drawn at the lower focus, so that the spirals appear reversed. All figures drawn from specimens fixed with sublimate-alcohol (Schaudinn) and stained with iron-haematoxylin (Heidenhain)—using, however, the haematoxylin in alcoholic solution.

PLATE X.

Trichonympha agilis.

- Fig. 1. *T. agilis* var. *japonica*, whole organism. ($4 \times \frac{1}{12}$.)
- Figs. 2–4. Anterior portion of *T. agilis* var. *japonica*. ($4 \times \frac{1}{12}$.)
- Fig. 5. *T. agilis* var. *formosana*, whole organism. ($2 \times \frac{1}{12}$.)
- Figs. 6–12. Stages of division of same. ($4 \times \frac{1}{12}$.)
- Fig. 13. Diagrammatic drawing showing structure of the anterior portion of *Trichonympha*. c, cap; a, axial core—hollow, showing wall and contents, and anterior terminal knob; i, inner layer of nipple wall; o, outer layer of nipple wall.

PLATE XI.

Pseudotriconympha grassii.

- Fig. 14. Whole organism. ($2 \times \frac{1}{12}$.)
- Fig. 15. Diagrammatic drawing of structure of the anterior portion.
- Figs. 16 and 17. Resting nuclei. ($4 \times \frac{1}{12}$.)
- Figs. 18–23. Stages of division. (Figs. 18–21, and 23, $4 \times \frac{1}{12}$; Fig. 22, $2 \times \frac{1}{12}$.)

PLATE XII.

Teratonympha mirabilis.

- Fig. 24. *T. mirabilis* var. *formosana*, whole organism. ($2 \times \frac{1}{12}$.)
- Fig. 25. Anterior portion of *T. mirabilis* var. *formosana*. ($4 \times \frac{1}{12}$.)
- Fig. 26. Anterior portion of *T. mirabilis* (type species). ($4 \times \frac{1}{12}$.)
- Figs. 27–31. Stages of division of *T. mirabilis*. ($4 \times \frac{1}{12}$.)

PLATE XIII.

Figs. 32–38. *Microspironympha porteri*.

(In Figs. 32, 34, 36, and 37, the specimens are drawn focused on lower surface, so that the spirals appear laetotropic, though they are really dextrotropic.)

- Fig. 32. Small individual. ($2 \times \frac{1}{12}$.)
- Figs. 33 and 34. Large individuals. ($2 \times \frac{1}{12}$.)
- Fig. 35. Diagrammatic drawing of the anterior portion.
- Figs. 36 and 37. Anterior portion of large individuals. ($2 \times \frac{1}{12}$.)
- Fig. 38. A late stage of division. ($2 \times \frac{1}{12}$.)

Figs. 39 and 40. *Holomastigotes elongatum* (?).

Fig. 39. Whole organism. ($4 \times \frac{1}{12}$.)

Fig. 40. Another specimen, showing the endoplasm and spiral ridges (really dextrotropic, but focused on lower surface). ($4 \times \frac{1}{12}$.)

Figs. 41 and 42. *Spirotrichonympha leidy*.

Fig. 41. Semi-diagrammatic drawing of the organism. ($4 \times \frac{1}{12}$.)

Fig. 42. Whole organism. ($2 \times \frac{1}{12}$.)

Figs. 43–52. *Holomastigotoides hartmanni*.

(In Figs. 43, 44, 45, 47, 48, and 51, the specimens are drawn focused on lower surface, so that the spirals appear reversed (laetotropic). They are really dextrotropic.)

Fig. 43. Whole organism. ($2 \times \frac{1}{12}$.)

Figs. 44–46. Anterior portion. ($4 \times \frac{1}{12}$.)

Figs. 47–51. Stages of division. ($4 \times \frac{1}{12}$.)

Fig. 52. Anterior portion of a young individual, soon after division. ($4 \times \frac{1}{12}$.)

Fig. 53. Diagrammatic drawings showing structure of body wall of *Teratonympha* (a), *Holomastigotoides* (b), and *Holomastigotes* (c).

PLATE XIV.

Pyrrsonympha.

(In Figs. 54, 55 and 66, the specimens are focused on lower surface, so that the spirals appear dextrotropic, though really laetotropic as in Fig. 65.)

Figs. 54 and 55. *P. grandis*, whole organisms. ($4 \times \frac{1}{12}$.)

Figs. 56 and 57. Anterior portion of *P. grandis*. ($4 \times \frac{1}{12}$.)

Figs. 58–61. Stages of division of *P. grandis*. (Figs. 58 and 60, $4 \times \frac{1}{12}$; Figs. 59 and 61, $2 \times \frac{1}{12}$.)

Figs. 62–64. Stages in degeneration of *P. grandis*. ($4 \times \frac{1}{12}$.)

Figs. 65 and 66. *P. modesta*; in the latter figure a screw-like form shown. ($4 \times \frac{1}{12}$.)

PLATE XV.

Dinenympha.

(In Figs. 68, 69, 71, 72, 79, and 81, the spirals are drawn reversely, the lower surface being focused. All spirals are really laetotropic.)

Figs. 67, 68. *D. exilis*, whole organisms. ($4 \times \frac{1}{12}$.)

Figs. 69, 70. *D. rugosa*, whole organisms. ($4 \times \frac{1}{12}$.)

Figs. 71 and 72. Middle portion of the body of *D. rugosa*, showing nuclei. More highly magnified.

Figs. 73 and 74. *D. nobilis*, whole organisms. ($4 \times \frac{1}{12}$.)

Fig. 75. Semi-diagrammatic drawing of the posterior portion of *D. nobilis*.

Figs. 76 and 77. *D. leidy*, whole organisms. ($4 \times \frac{1}{12}$.)

Fig. 78. *D. parva*, whole organism. ($4 \times \frac{1}{12}$.)

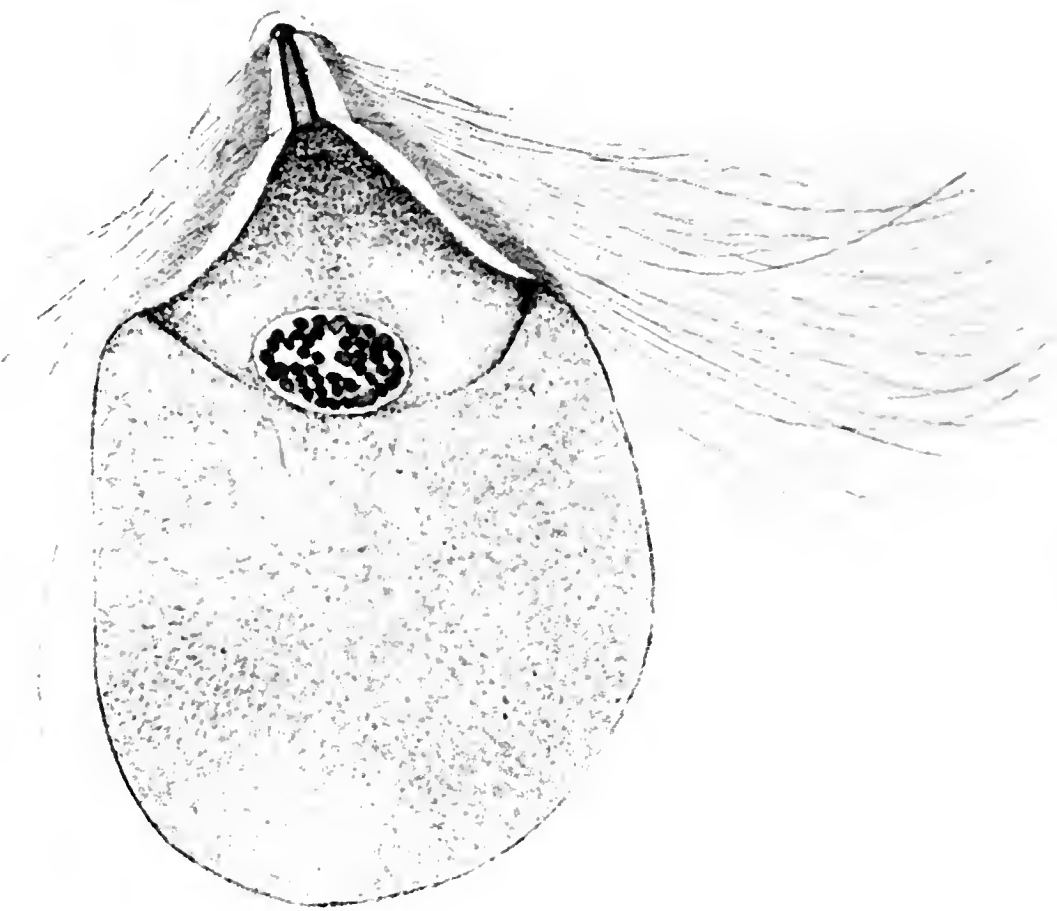
Fig. 79. *D. porteri*, type 1. ($4 \times \frac{1}{12}$.)

Fig. 80. *D. porteri*, type 2. ($4 \times \frac{1}{12}$.)

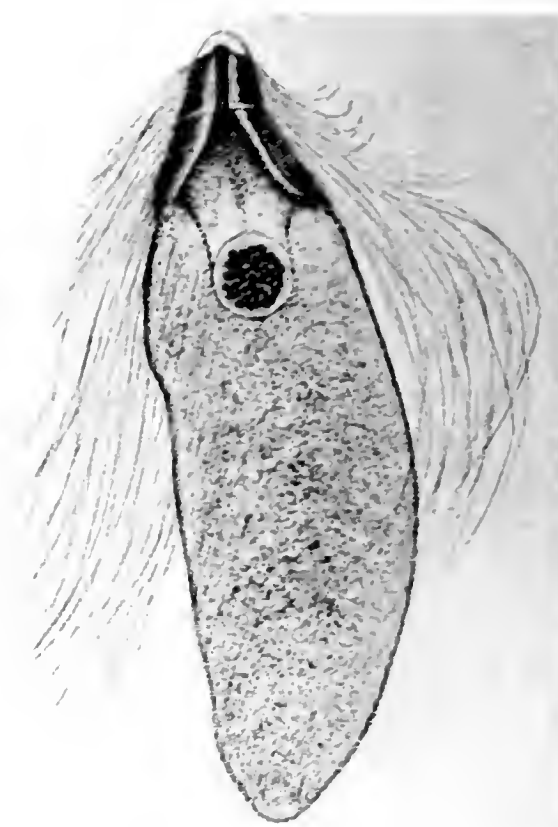
Fig. 81. *D. porteri*, type 3. ($4 \times \frac{1}{12}$.)

Fig. 82. Semi-diagrammatic drawing of the posterior end of *D. porteri*, type 1.

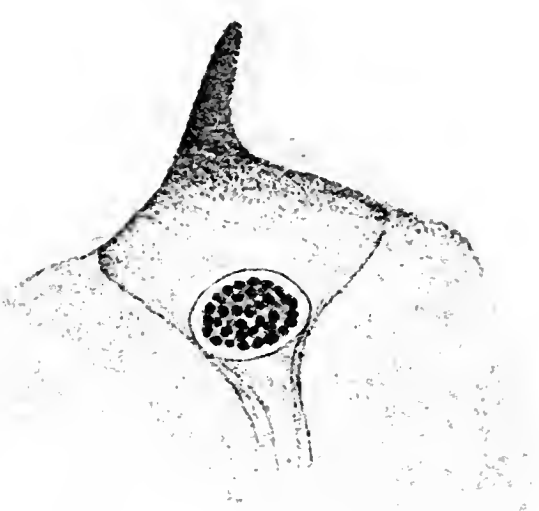
Figs. 83 and 84. Stages of division of *D. porteri*. ($4 \times \frac{1}{12}$.)



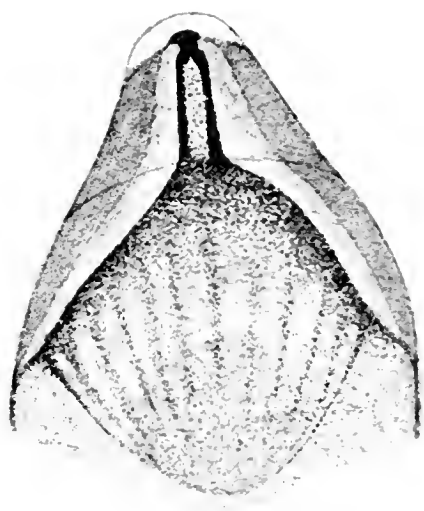
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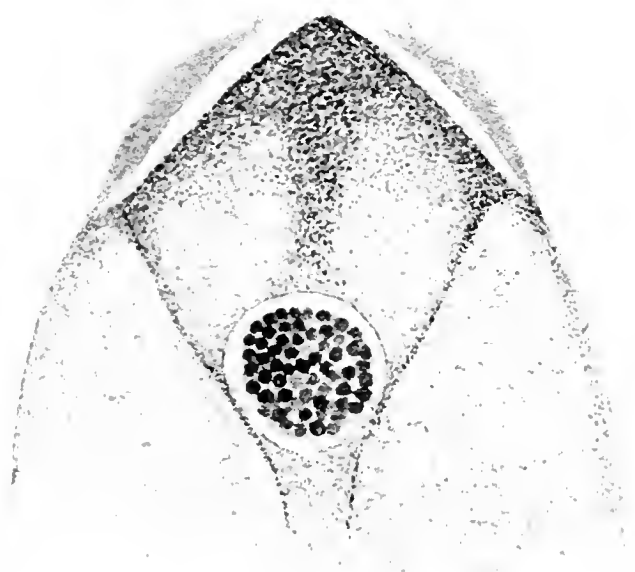
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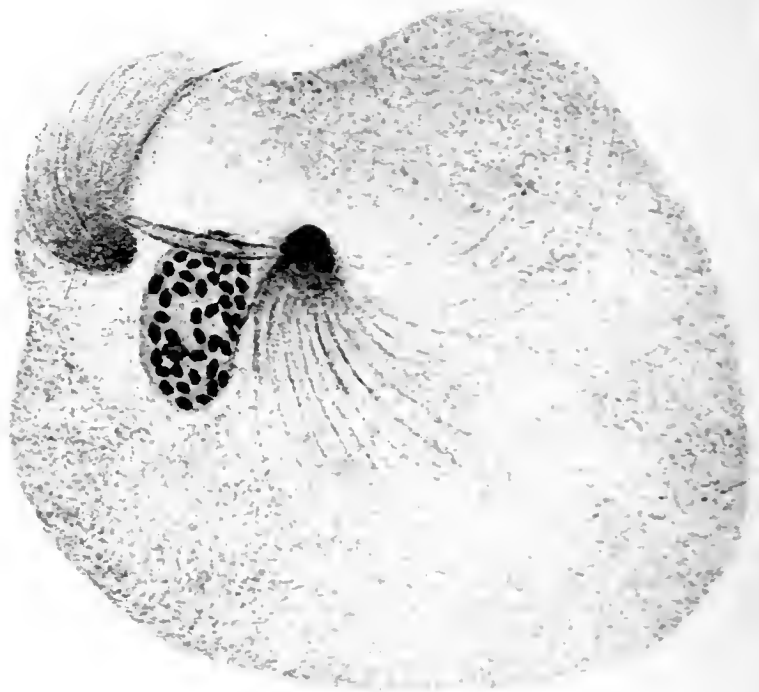
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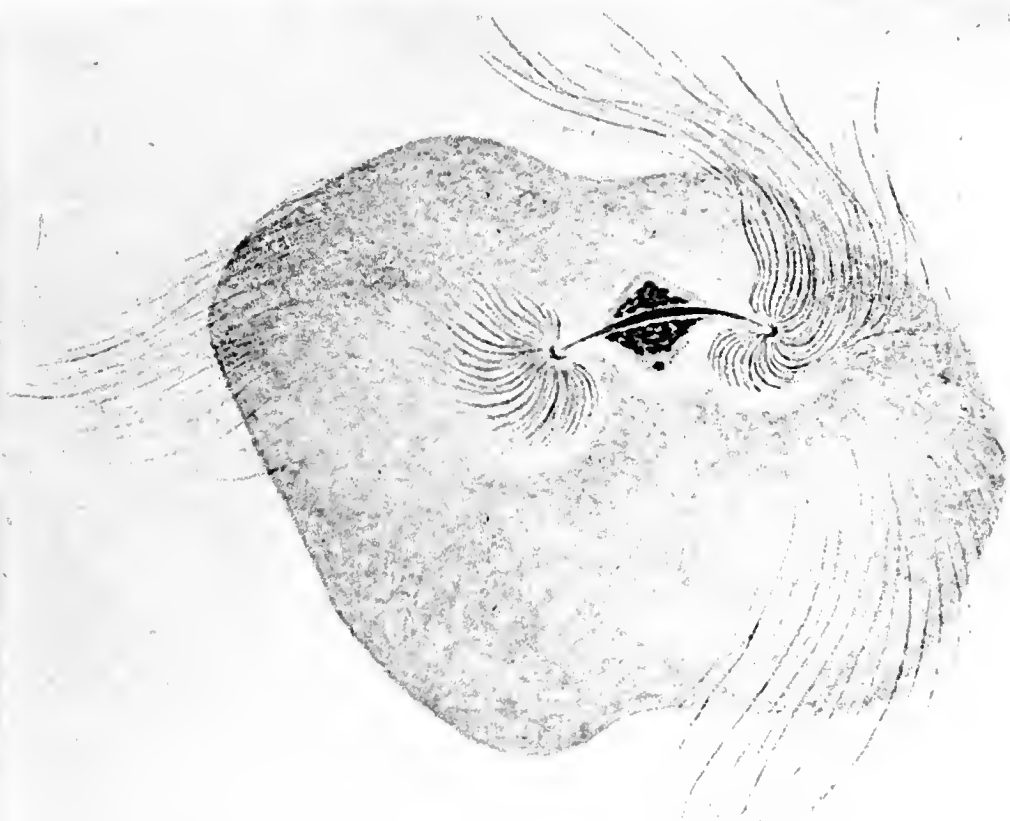
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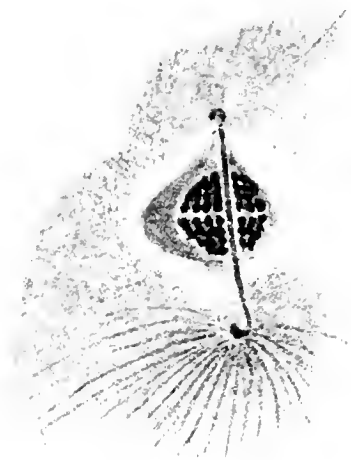
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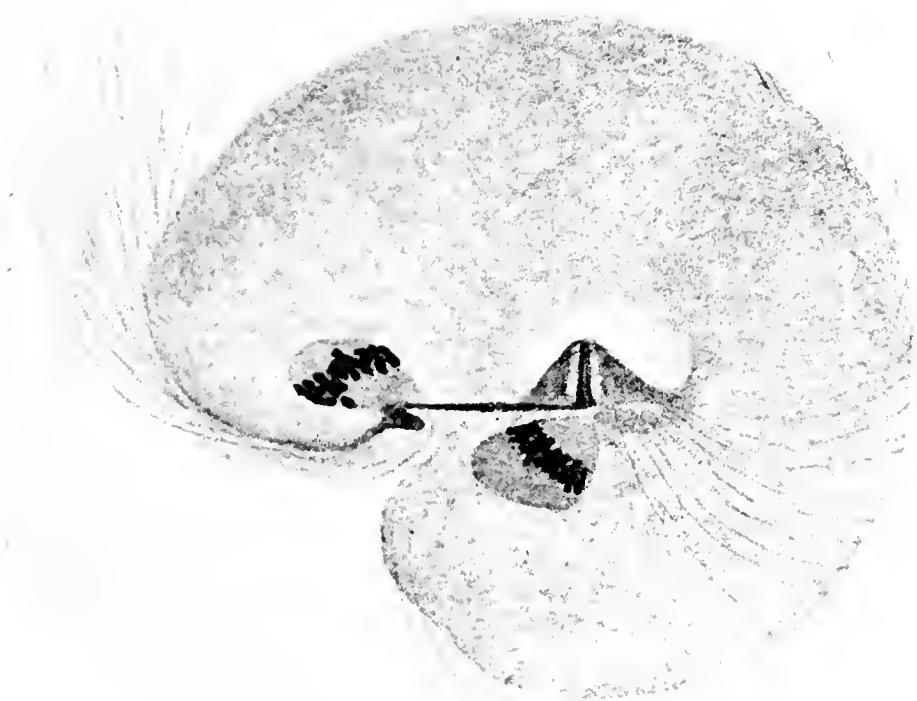
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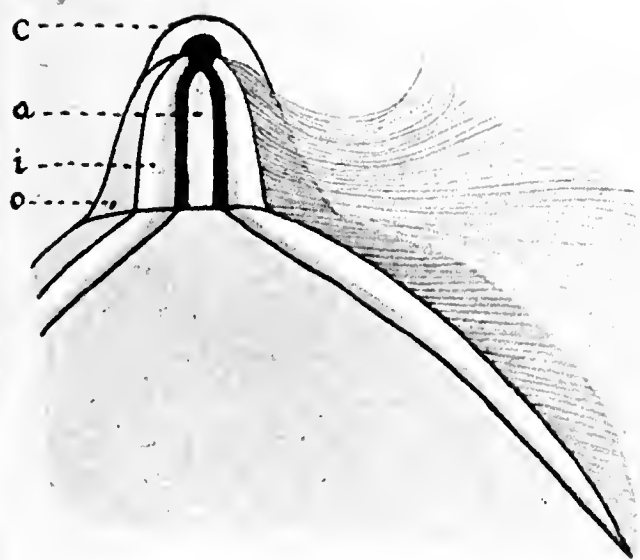
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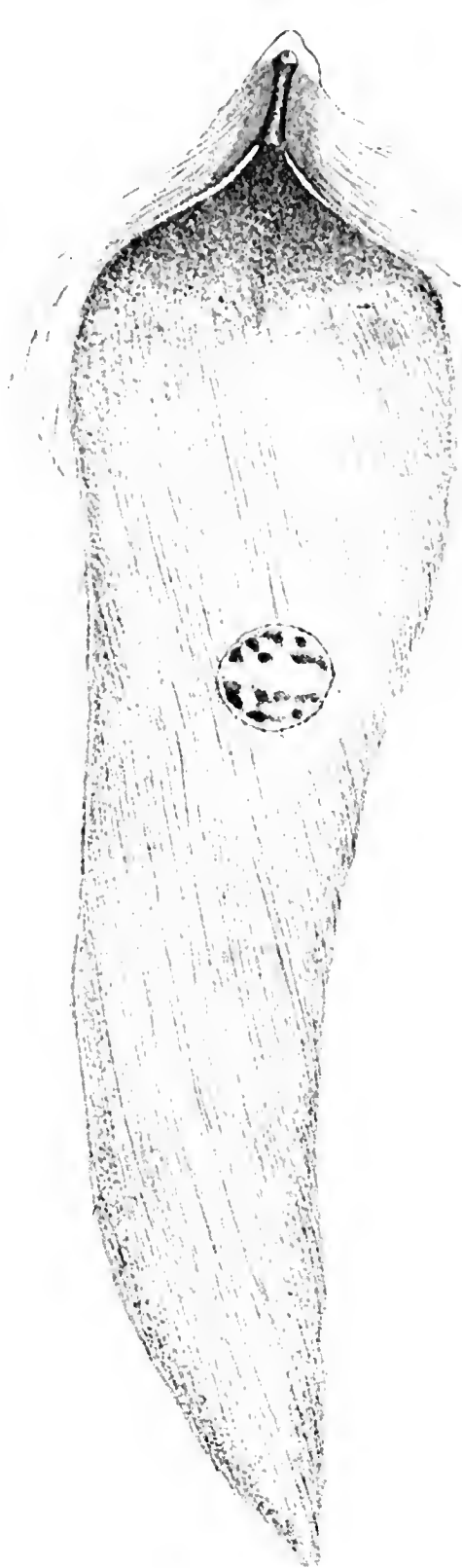
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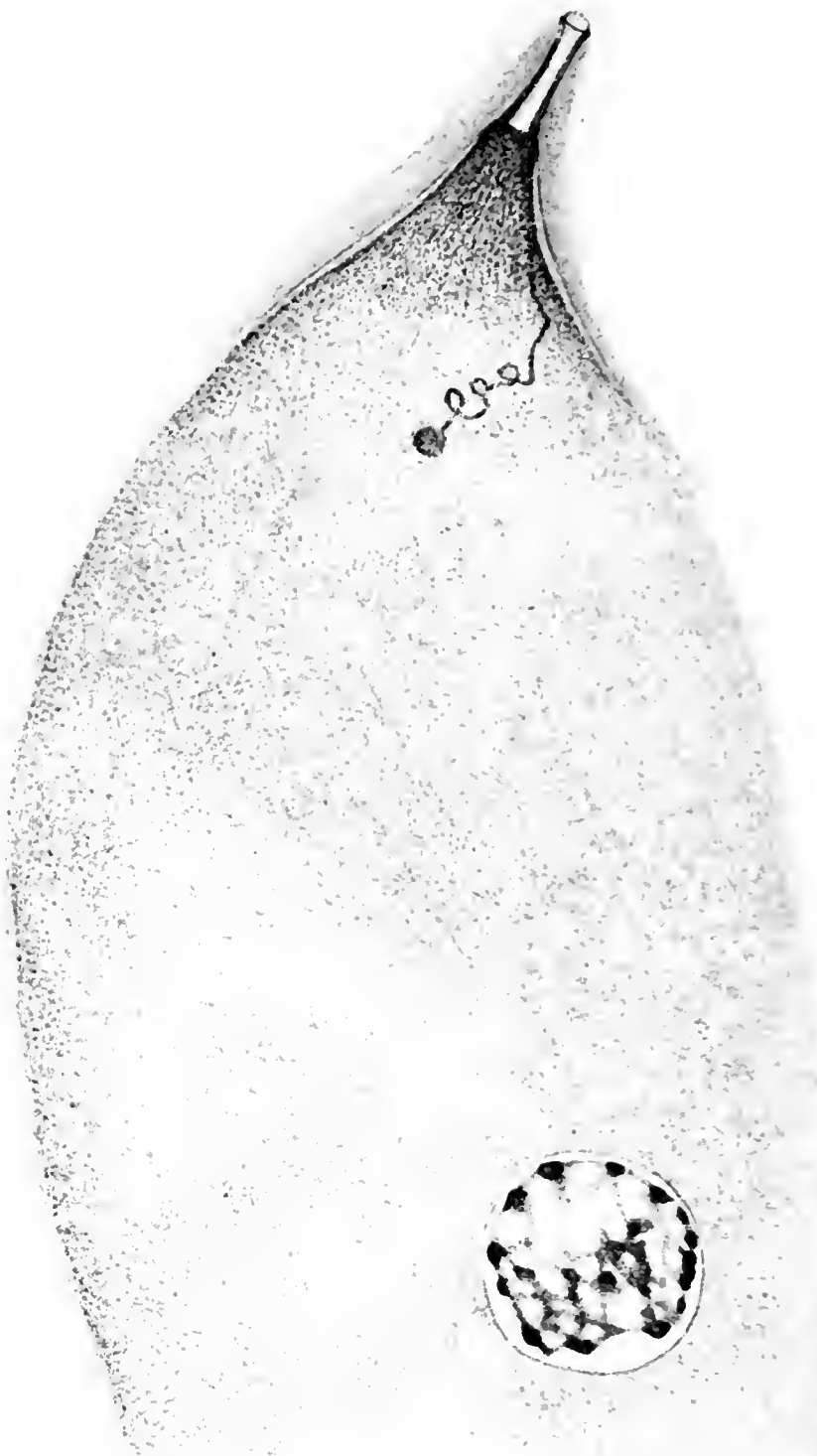
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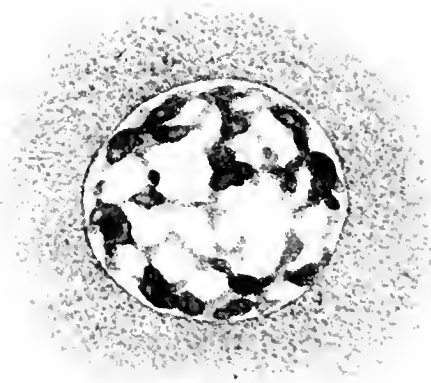
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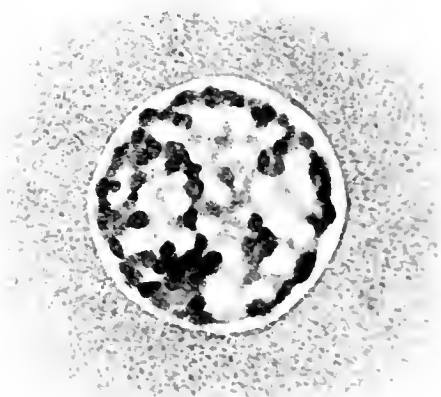
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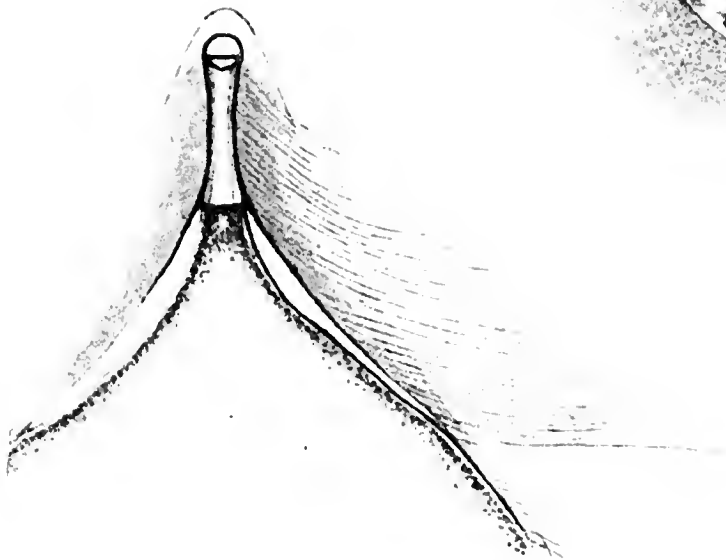
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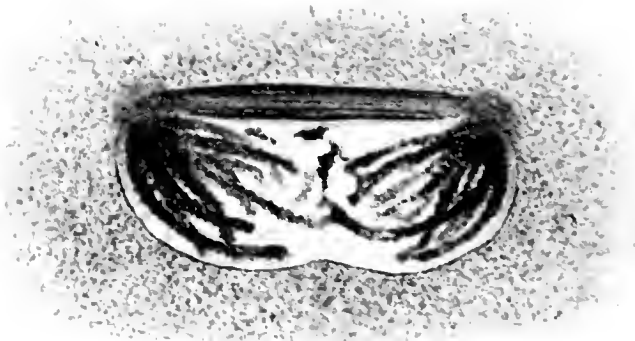
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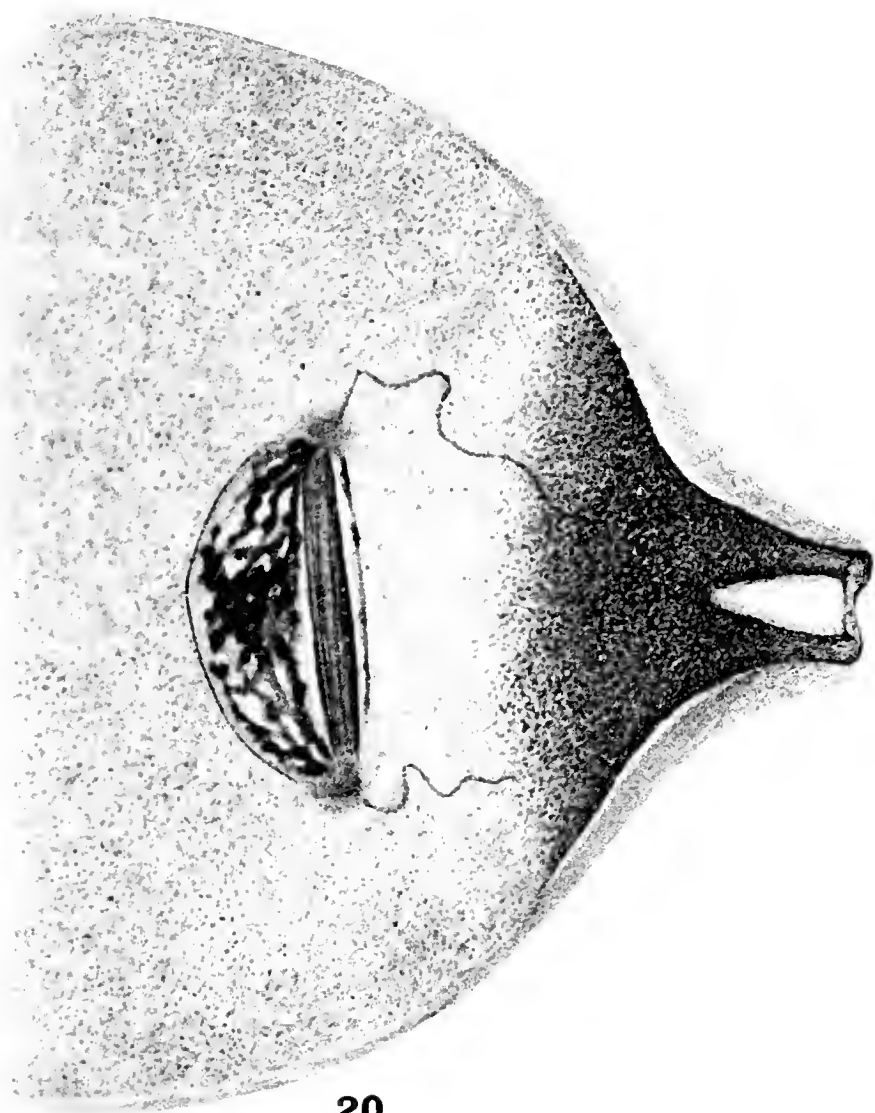
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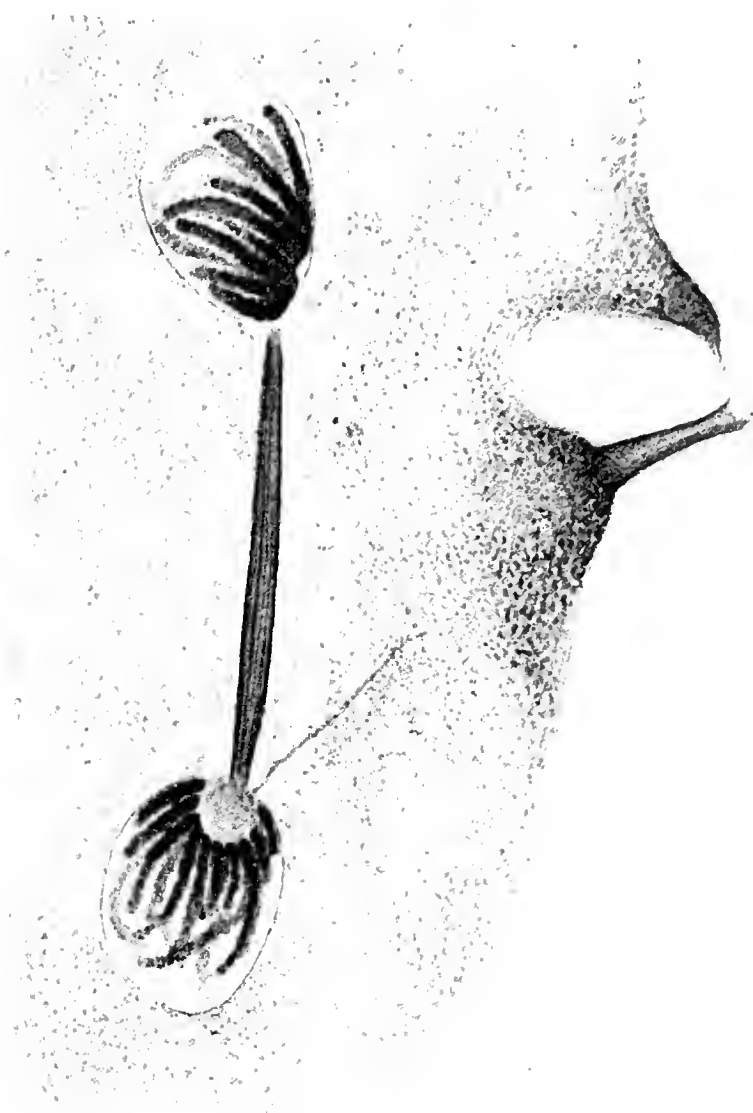
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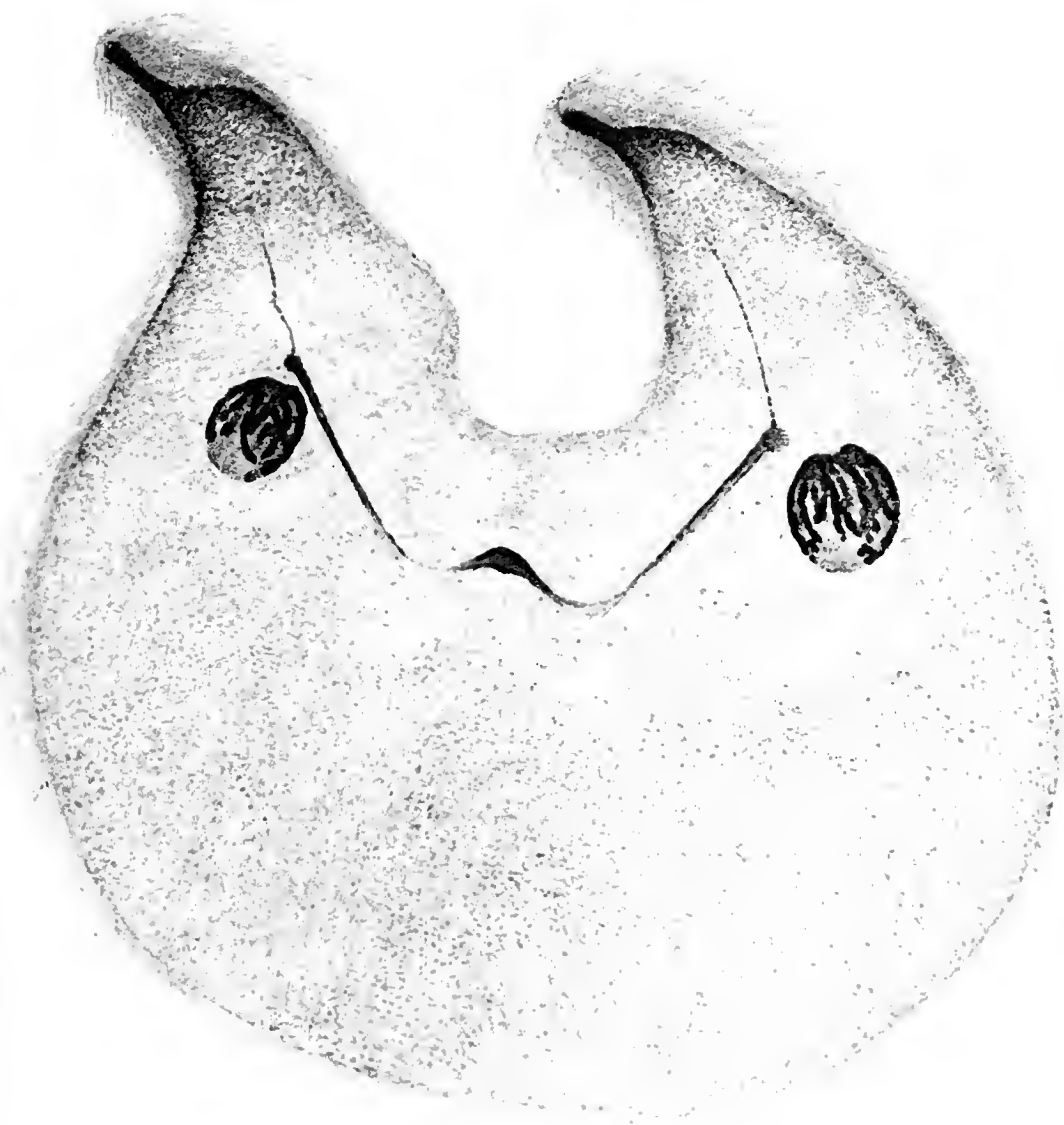
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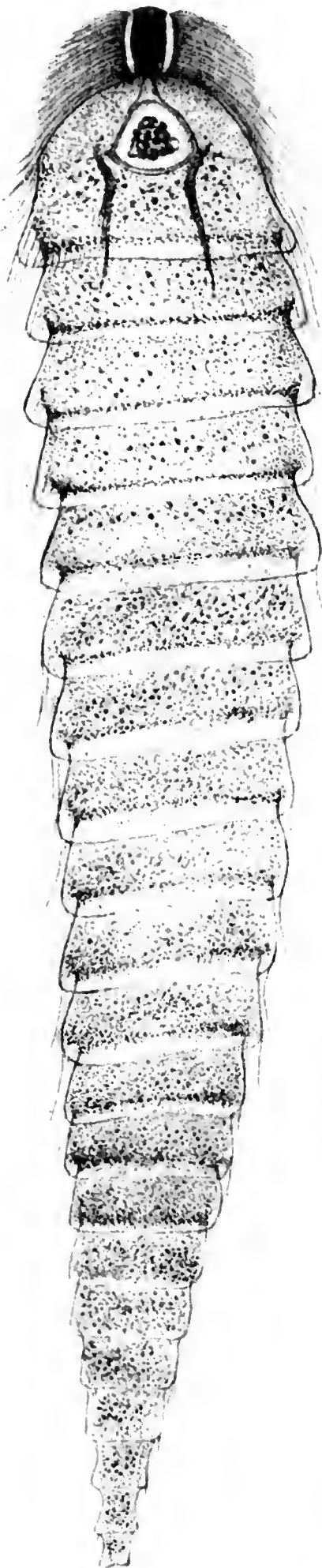
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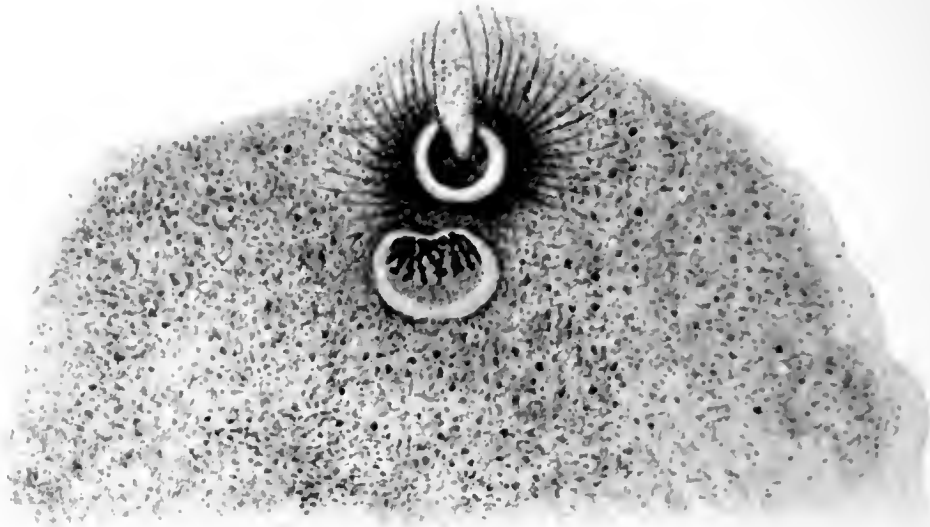
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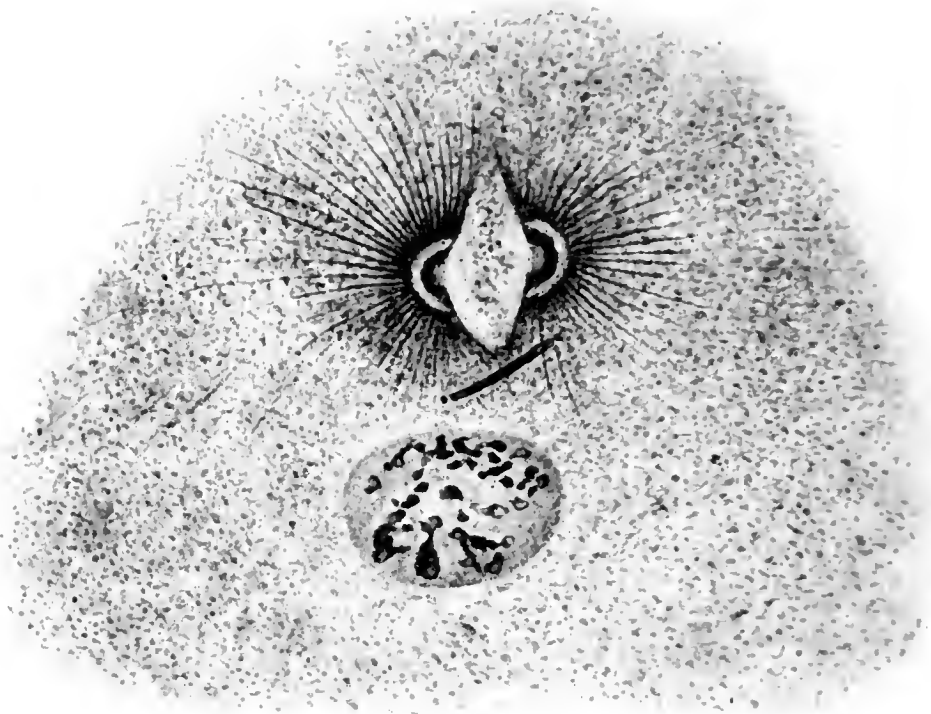
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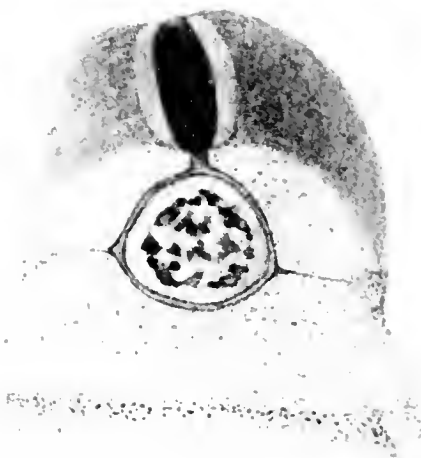
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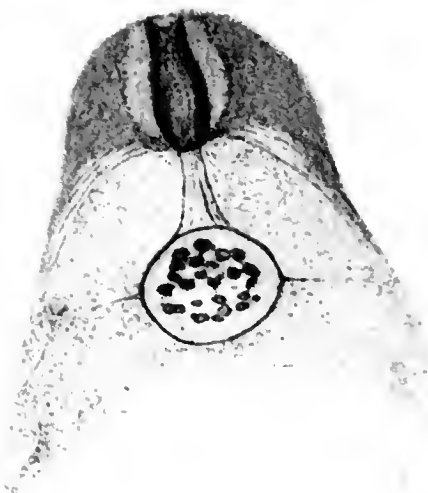
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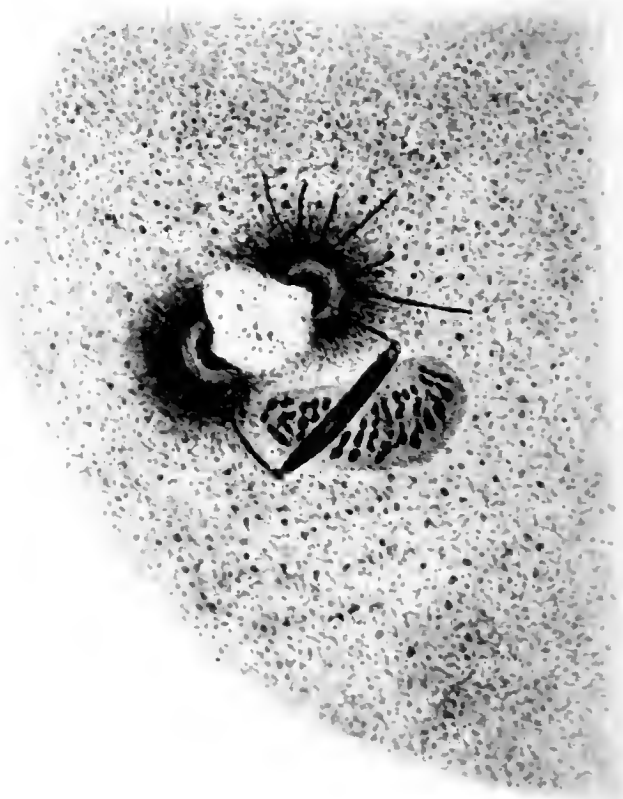
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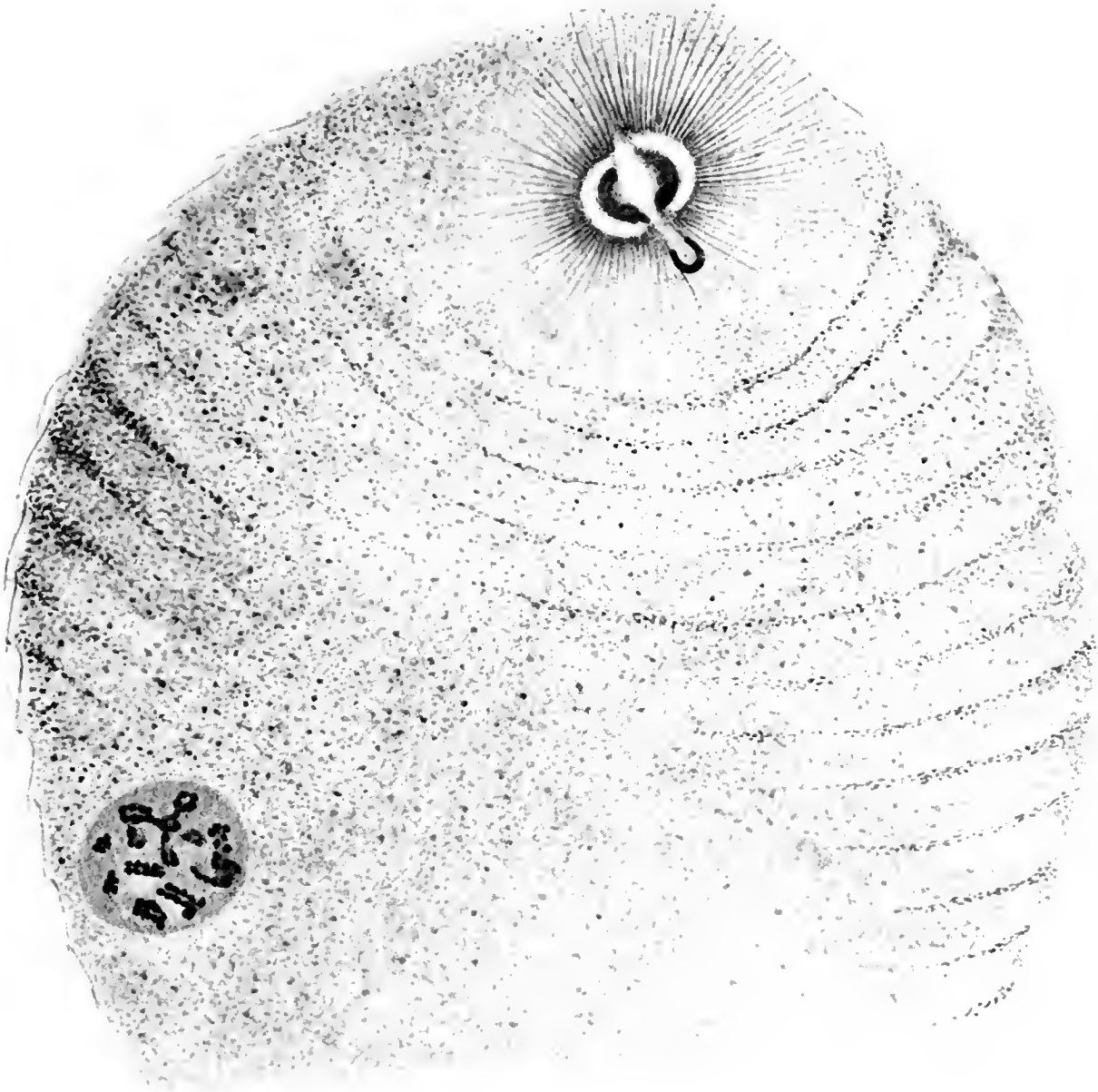
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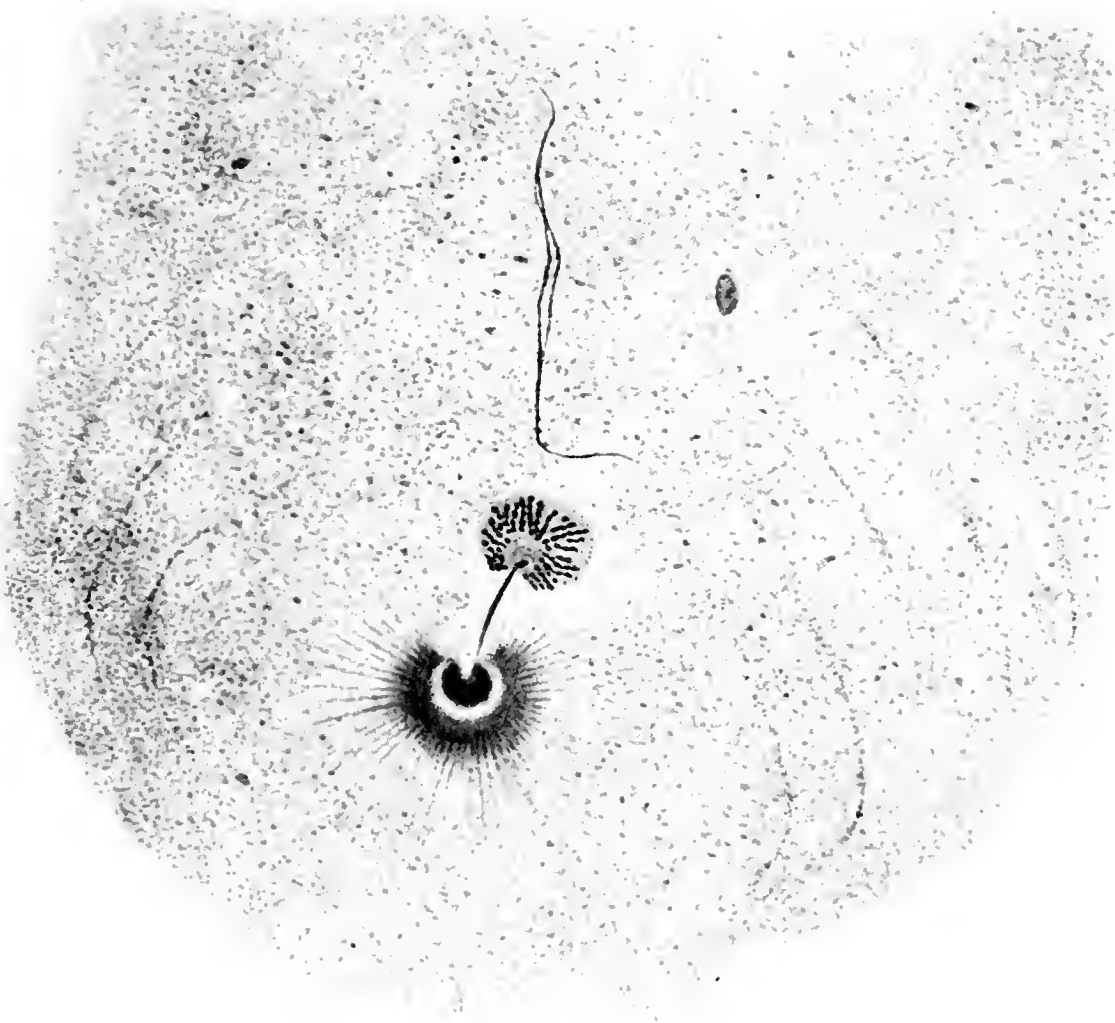
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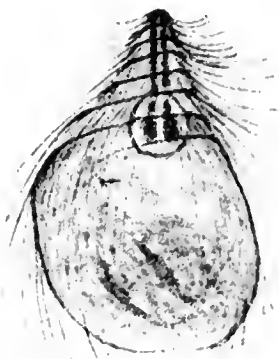
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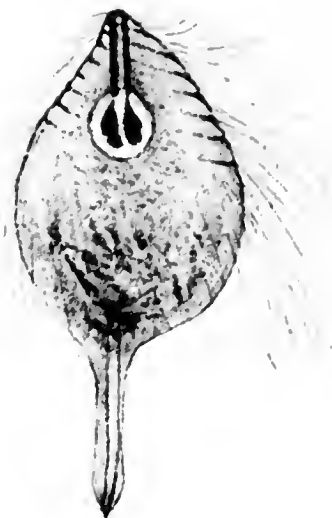
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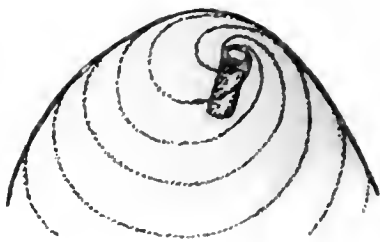
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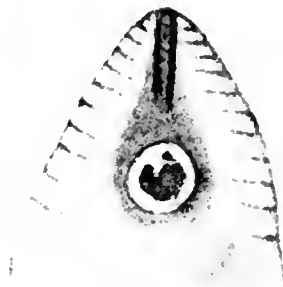
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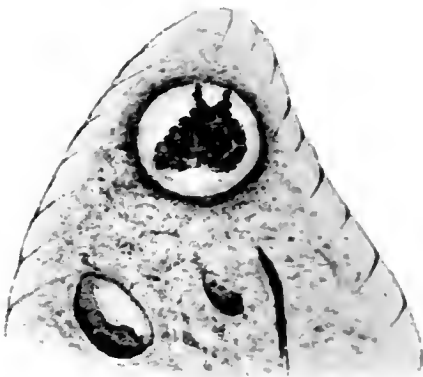
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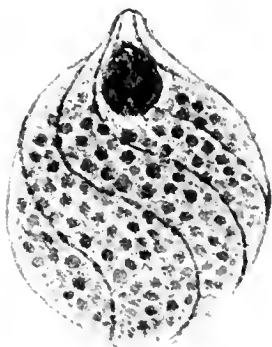
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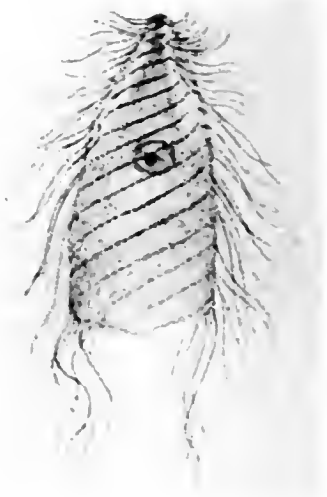
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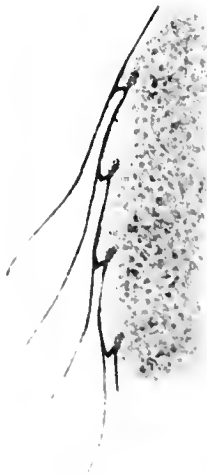
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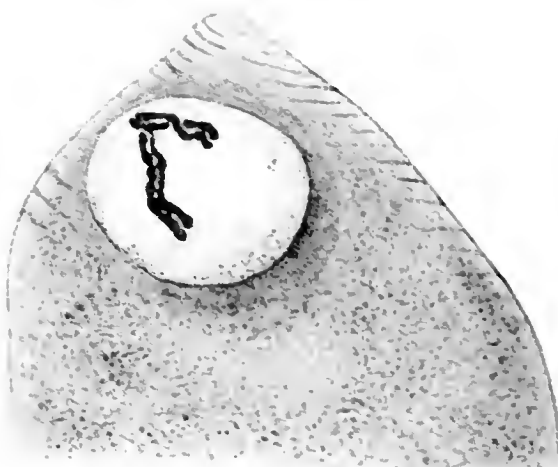
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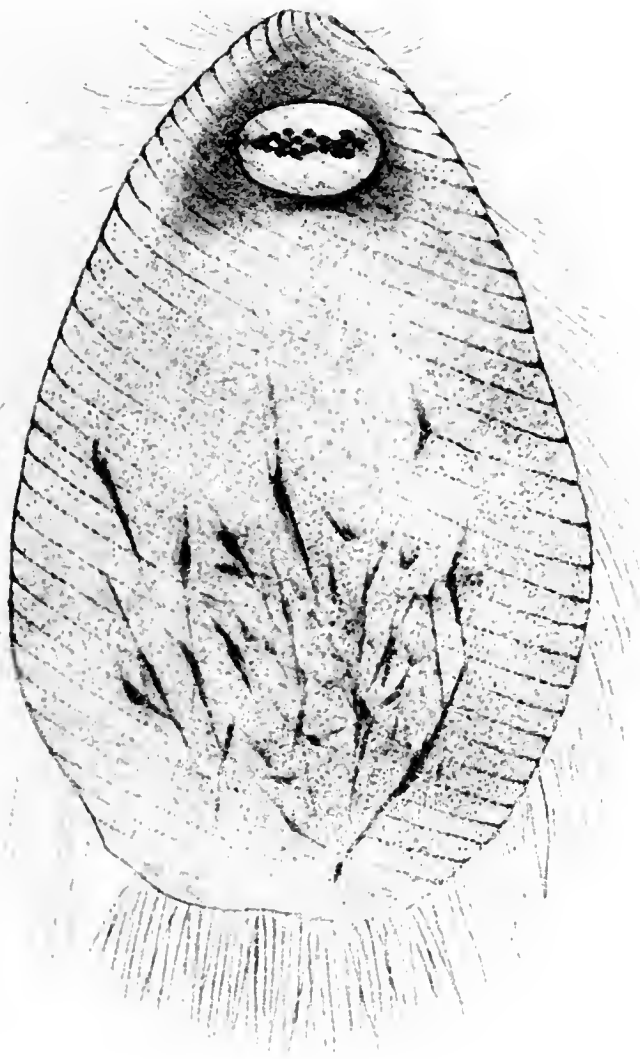


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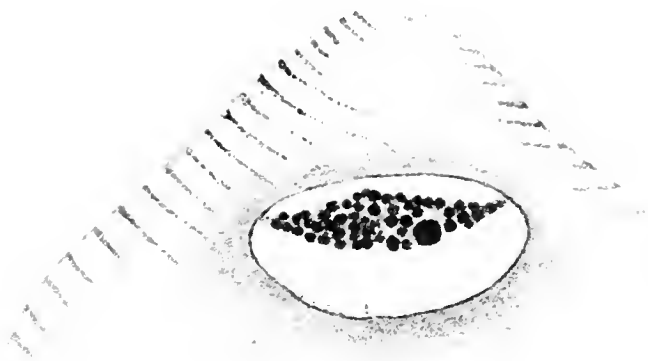
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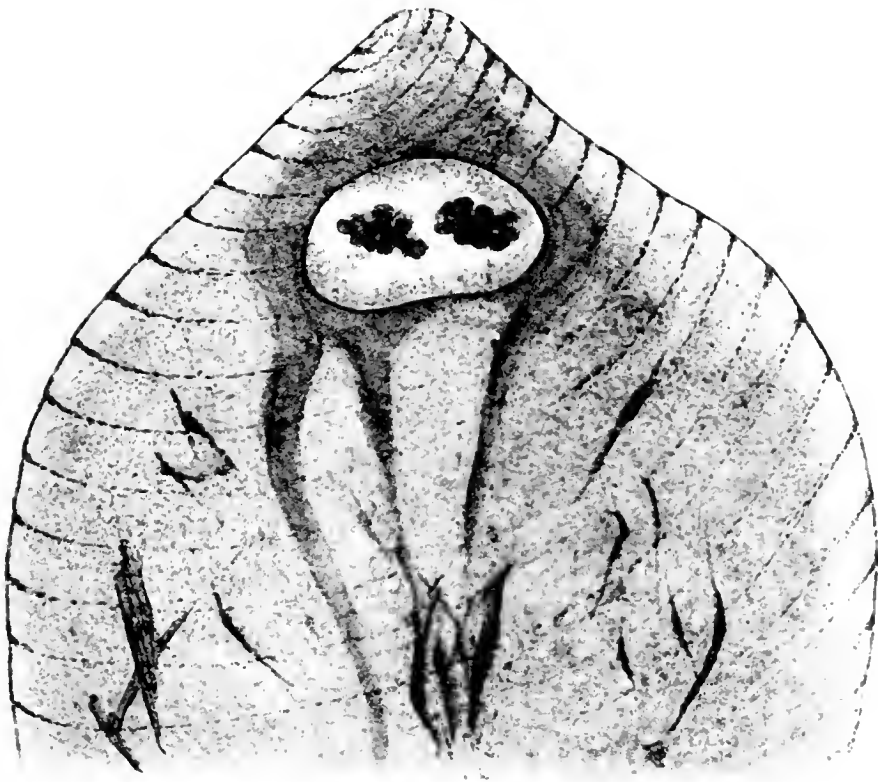
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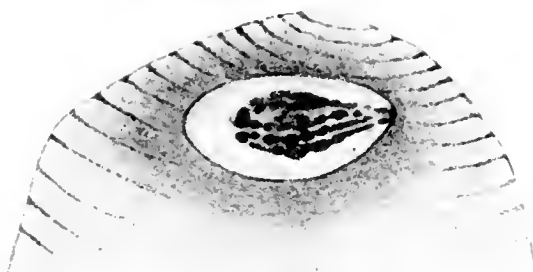
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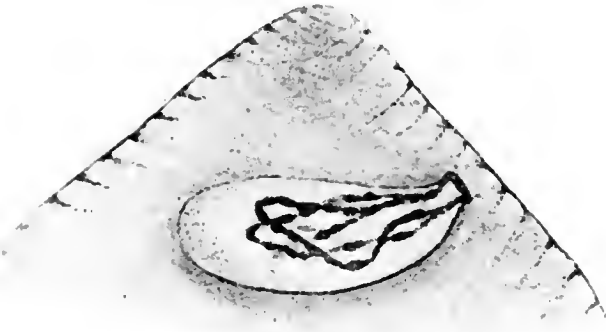
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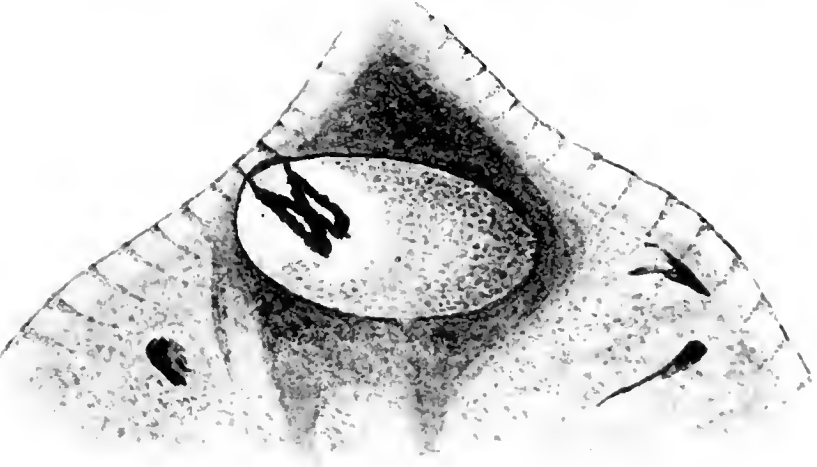
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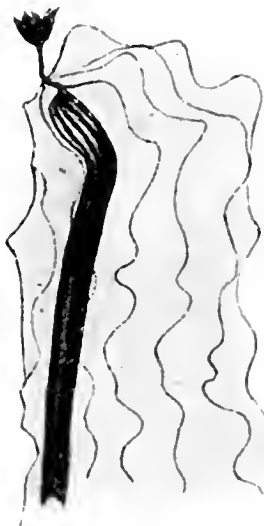
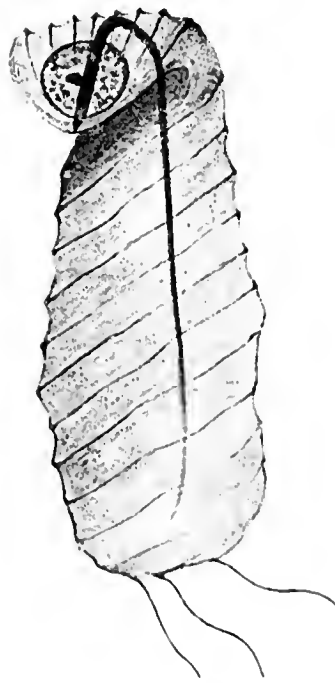
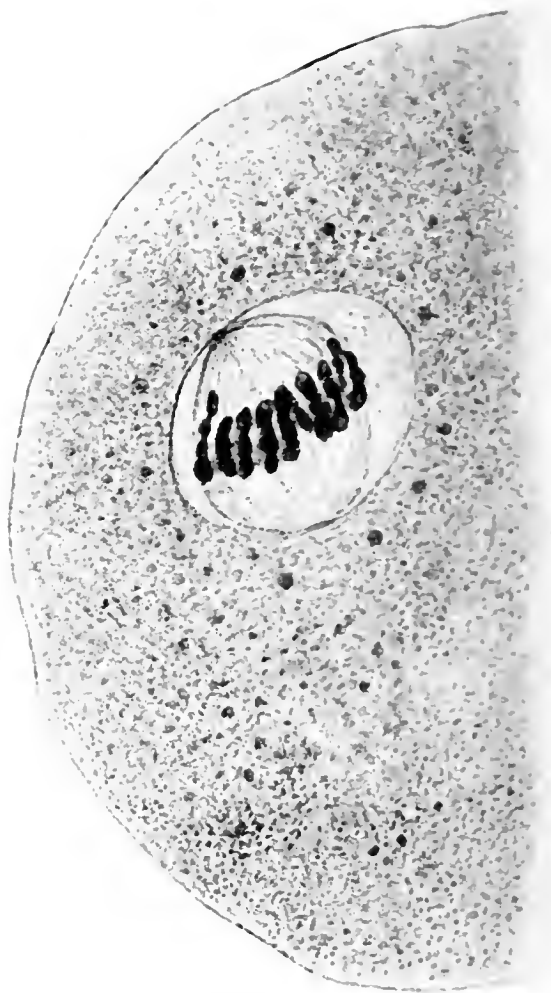
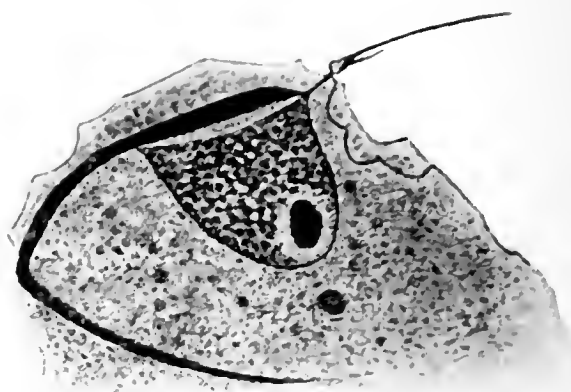
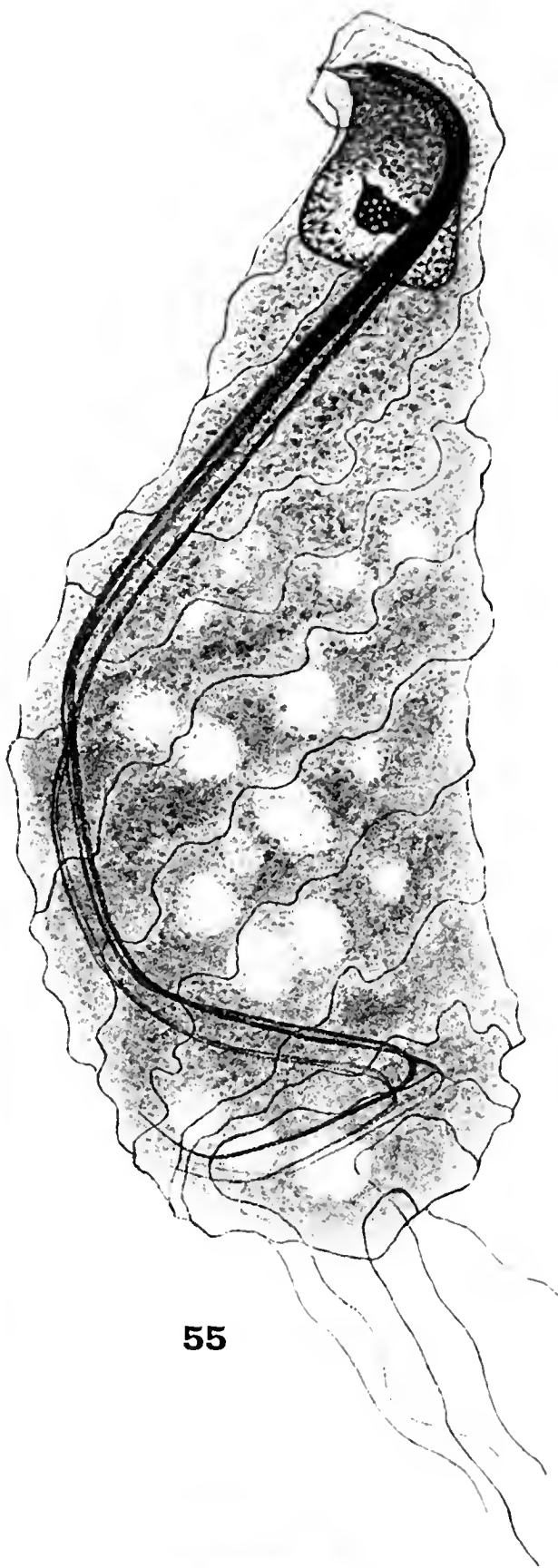
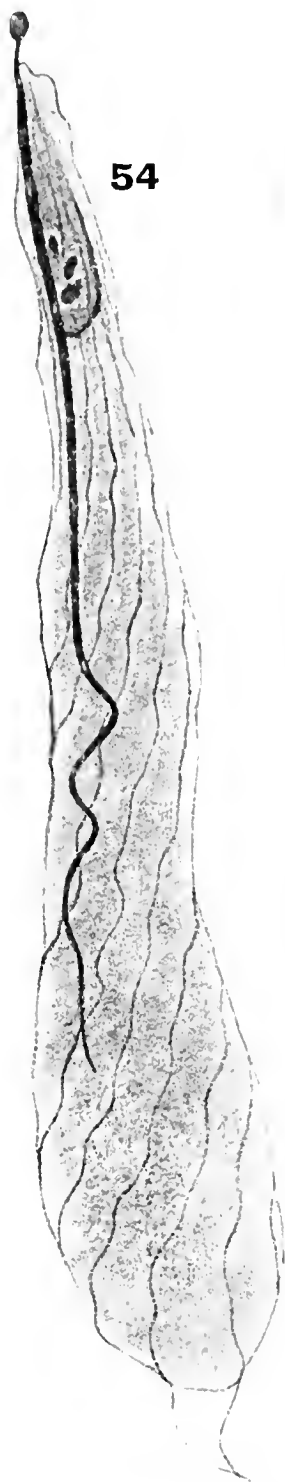
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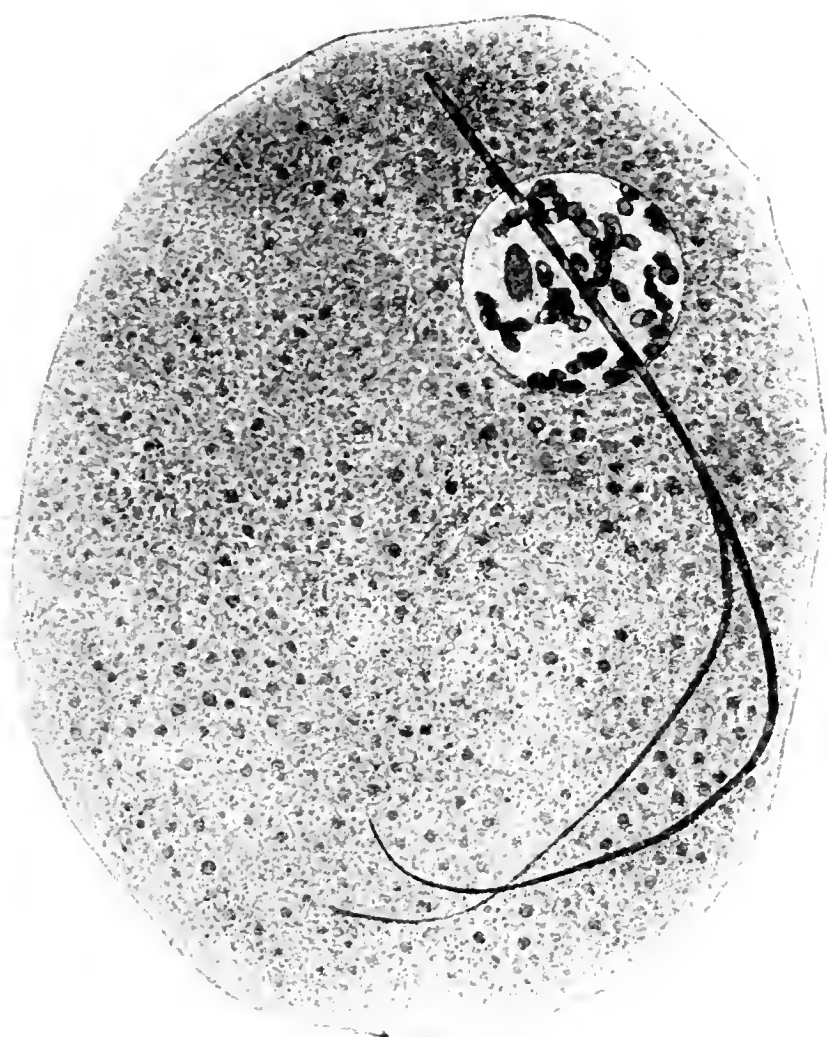


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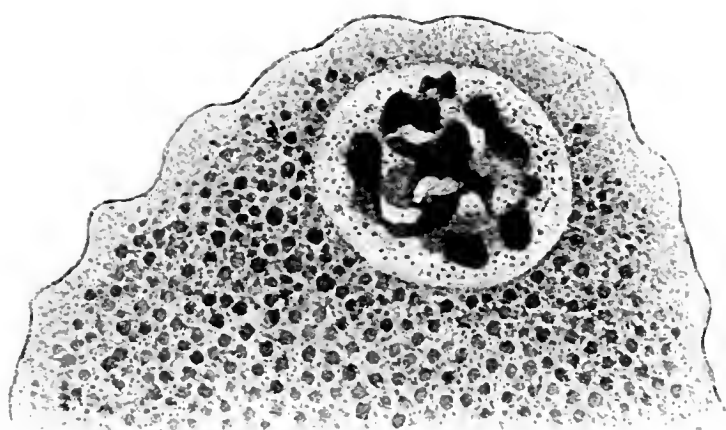




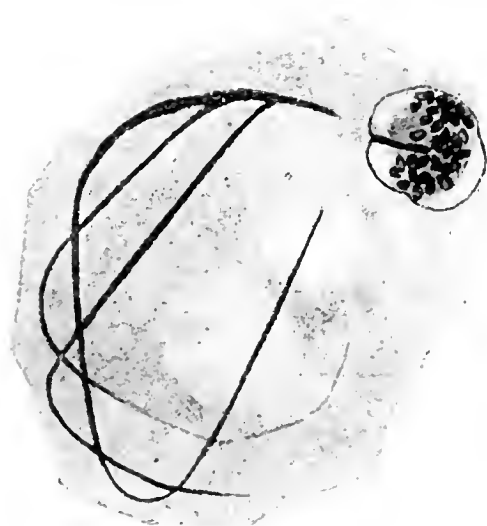
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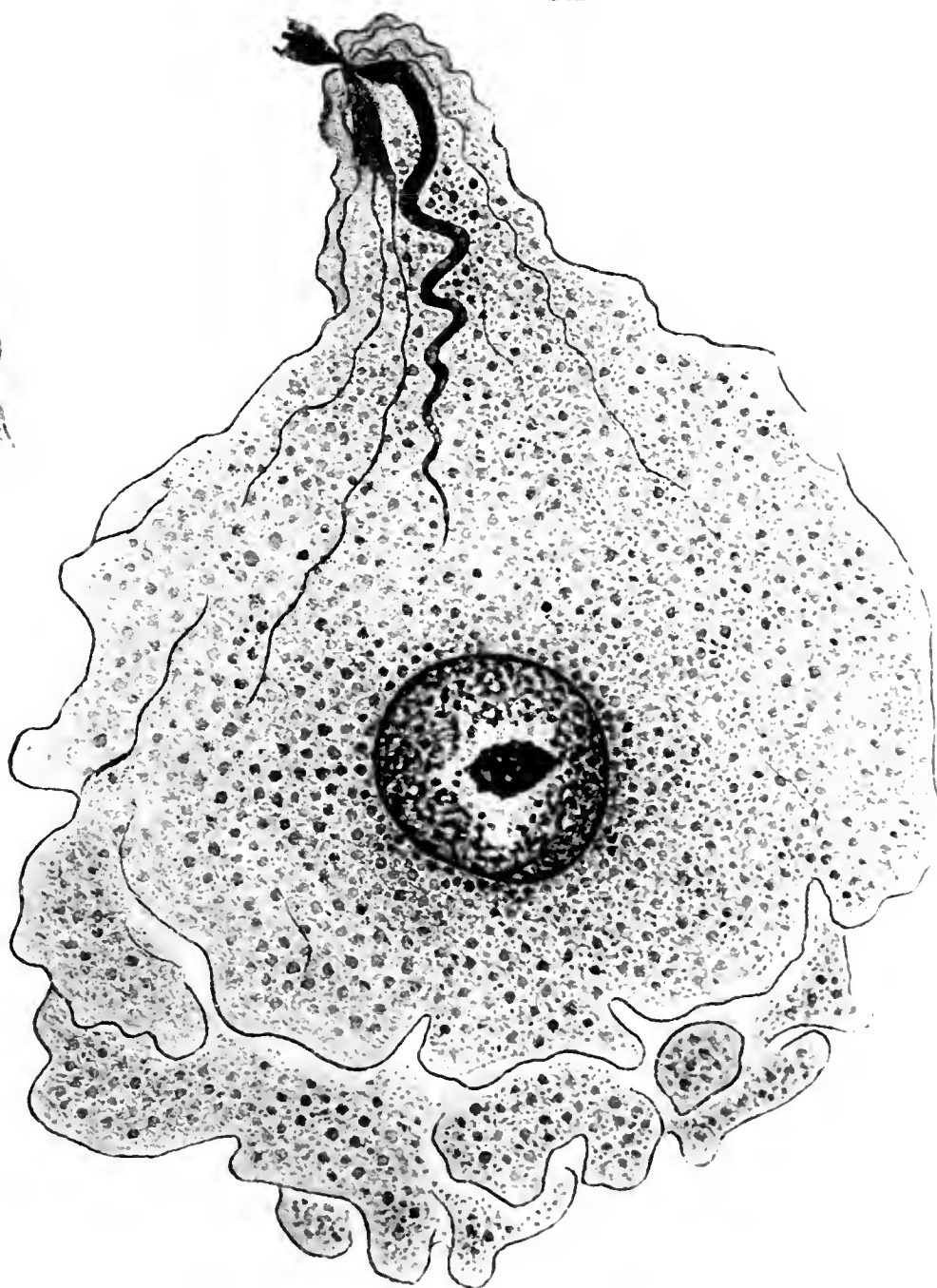
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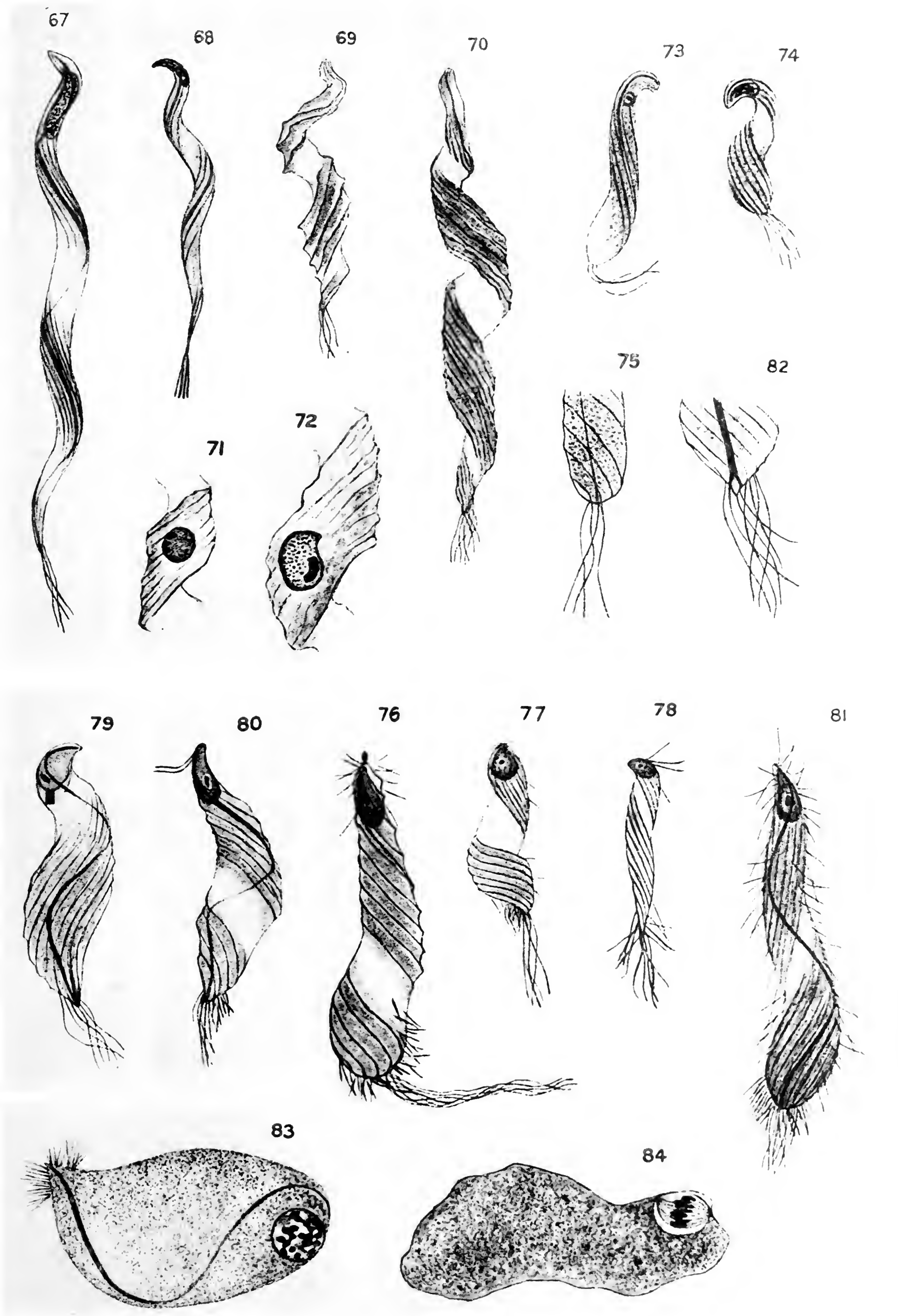
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RICHARD OWEN

1804—1892

From the picture by H. G. Pickersgill, R.A. (1845), in the National Portrait Gallery
Photographed by Messrs Emery Walker, Ltd., London



ATHANASIVS KIRCHER

1601—1680



HON. ROBERT BOYLE

1627—1691

ON SOME NEMATODE PARASITES OF THE CAMEL IN INDIA.

BY C. L. BOULENGER, M.A., D.Sc.,

Professor of Zoology, University of the Punjab, Lahore.

(From the Punjab Veterinary College Laboratories.)

(With 3 Text-figures.)

ON A NEW CESTODE FROM THE POUCHED RAT, *CRICETOMYS GAMBIANUM*.

BY F. J. MEGGITT, M.Sc., PH.D.

A Correction.

It is regretted that, in acknowledging the receipt of specimens of the species described under this title in *Parasitology*, XIII, p. 194, they were erroneously stated to have been received from Professor Nuttall. The specimens in question were collected by Mr W. Mansfield-Aders, Government Biologist, Zanzibar, and sent for identification through Dr C. L. Boulenger. The confusion arose through the circumstance that we have received specimens repeatedly from Professor Nuttall and that the latter has also received specimens from Mr Mansfield-Aders. I have much pleasure in taking this opportunity of rectifying the mistake and expressing my thanks to Mr W. Mansfield-Aders.

N. spathiger (1896) must have been based in part on females of another species which he names *N. dromedarii*. *N. spathiger* is also shown to occur in other ruminants (*e.g.* sheep, cattle, etc.) having been confused with the type species *N. filicollis* (Rud.).

It was therefore to be expected that the worms from the camel in the Punjab would be referable to one of these three forms, the specimens before me are, however, shown in this paper to belong to a fourth species, *N. mauritanicus* Maupas and Seurat (1912) hitherto known from North Africa only.

Haemonchus longistipes, Railliet and Henry, 1909.

SPECIFIC DIAGNOSIS. *Haemonchus*¹: Head, 0.03–0.033 mm. in diameter. Buccal lancet well developed, 0.01 mm. long, sometimes seen projecting through the mouth-opening.

Cervical spines, 0.39–0.53 mm. from the anterior extremity of the body.

Oesophagus, 1.6–2.1 mm. in length. Diameter of body at the base of the oesophagus, 0.2–0.35 mm.

Male, 18–25 mm. long. Maximum thickness about 0.4 mm. Lateral lobes of the bursa very large, 0.8–1.2 mm. long, with a width of about 0.55 mm.

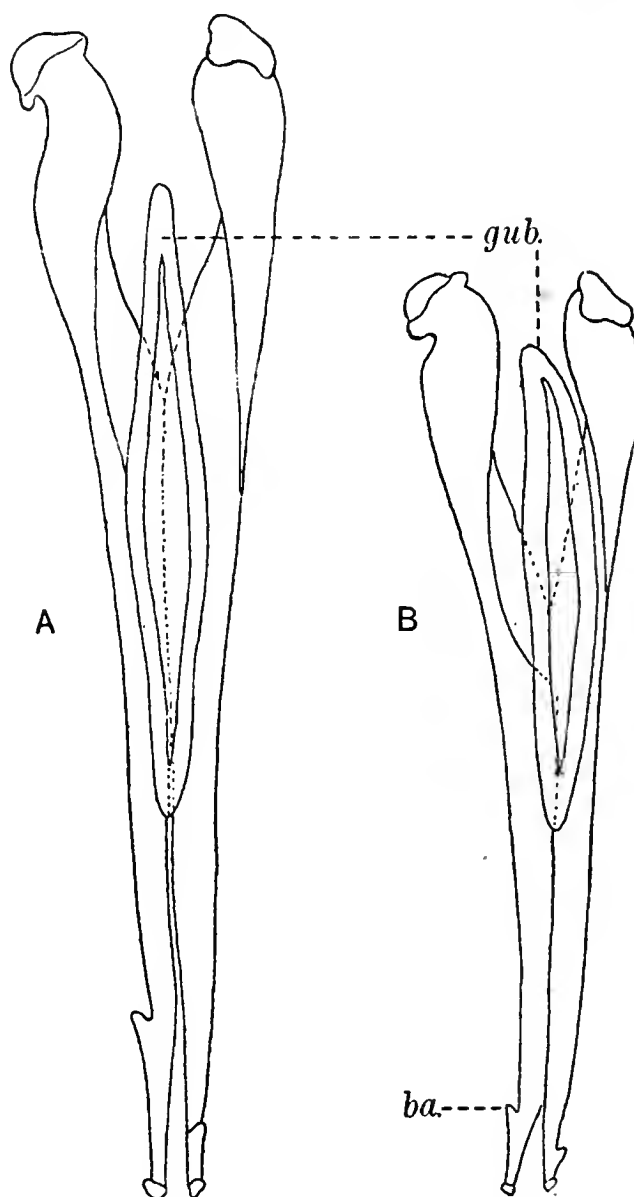


Fig. 1. Spicules and gubernaculum of male: A. *Haemonchus longistipes*; B. *Haemonchus contortus*.
× 150.

The asymmetrical dorsal lobe is also large, measuring 0.27–0.32 mm. long by about 0.15 mm. wide. The stem of the dorsal ray has a length at least twice that of its branches.

Spicules, 0.6–0.65 mm. long, with barb-like projections posteriorly; the barb of the right spicule situated 0.09–0.1 mm., that of the left spicule, 0.038–0.04 mm., from the posterior end (Text-fig. 1).

Gubernaculum 0.3–0.33 mm. long, as in the type species fusiform, with thickened edges.

¹ For the generic diagnosis *v.* Ransom, 1911, p. 49; to facilitate comparison with the type species the specific diagnosis of *H. longistipes* is given in the same form as that used by Ransom.

The cloaca is provided with a large ventral lip (Text-fig. 2).

Female, 23–35 mm. long, with a maximum thickness of 0.45–0.65 mm.

Vulva, a transverse slit, 4.5–6.8 mm. from the posterior extremity of the body; it is not covered by a large, linguiform process as in the type species, that structure being replaced by a much shorter conical projection of the body-wall, situated to one side of the genital opening (Text-fig. 3).

Ovijectors, 0.8–1.1 mm. long. Ovaries wound spirally around the intestine as in *H. contortus*.

Anus, 0.47–0.58 mm. from the tip of the slender, pointed tail.

Eggs, 0.068–0.07 mm. long by 0.04–0.05 mm. wide, segmenting when laid.

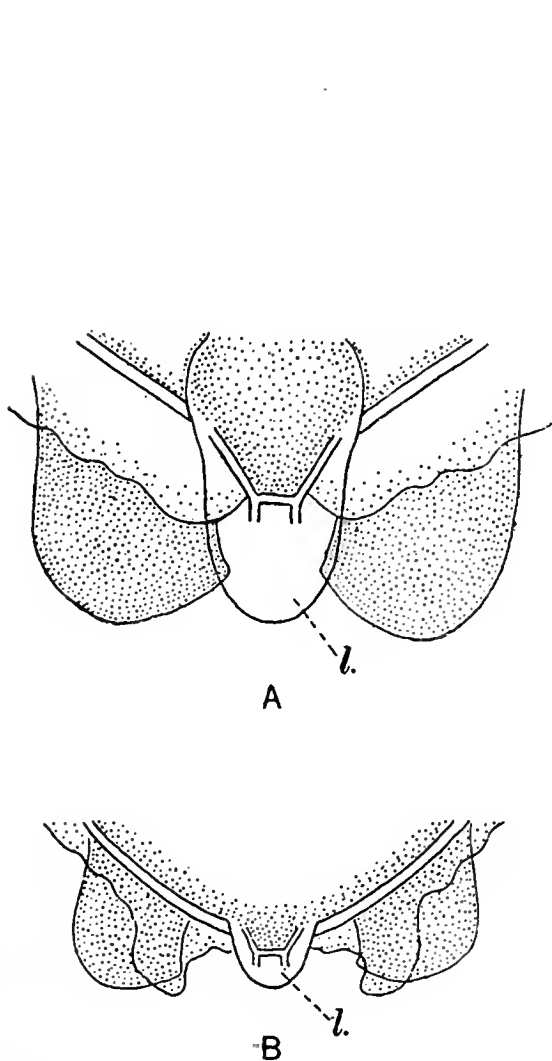


Fig. 2. Cloacal region of male: A. *Haemonchus longistipes*; B. *Haemonchus contortus*. $\times 250$.

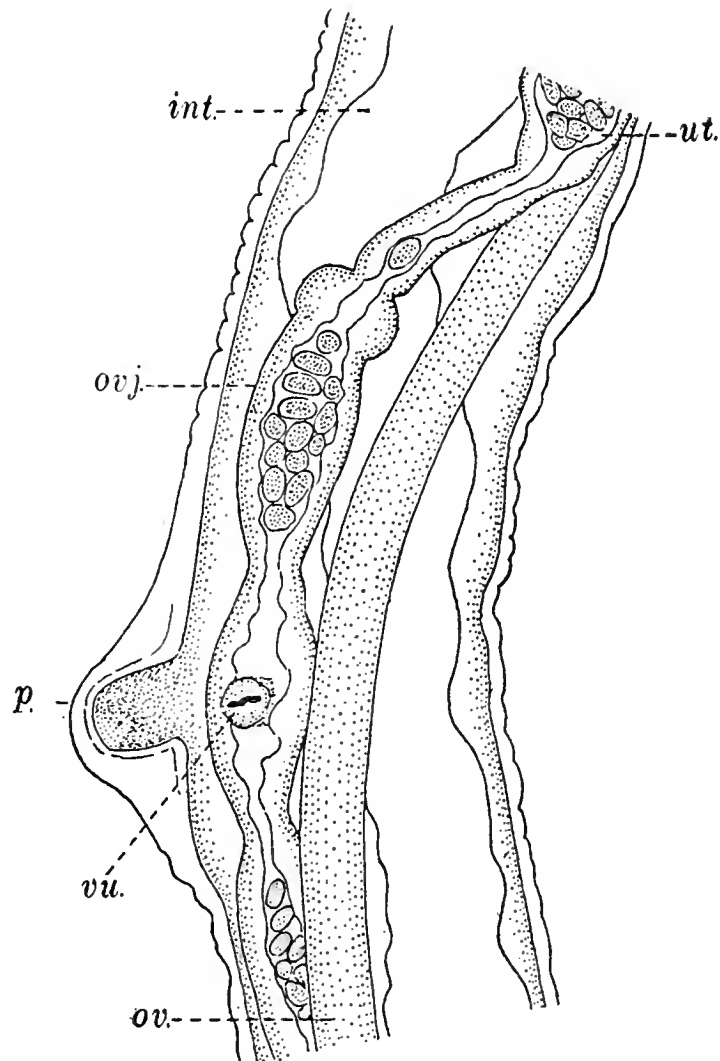


Fig. 3. *Haemonchus longistipes*, Body of female in region of vulva. $\times 60$.

LOCATION. Fourth stomach of the camel (*Camelus dromedarius*). Africa and India.

The measurements given above show that *Haemonchus longistipes* is a somewhat larger form than the type species. The males may be distinguished from those of *H. contortus* by the character of the posterior ray of the bursa, by the size of the cloacal lip and by the length of the spicules, as well as the position of the barbs at the posterior extremities of these structures. The females of *H. longistipes* may be recognized by the absence of the linguiform process over the vulva in fully developed specimens, and by the measurements of the eggs, which are smaller than those of the type species.

***Nematodirus mauritanicus*, Maupas and Seurat, 1912.**

Two female specimens of this species were found, labelled *Nematodirus spathiger* Railliet, in the collections of the Punjab Veterinary College. This form has not been previously described outside Africa, worms of the genus *Nematodirus* from the camel in N. India having been regarded as belonging to Railliet's species.

In the Punjab, as elsewhere, a number of species have probably been confused under the name *N. spathiger*; it would therefore be desirable to examine a larger series of specimens from the camel in this province; during my stay in Lahore I was unfortunately unable to obtain more material.

The following measurements and brief description show that the specimens observed by me agree well with the account of *N. mauritanicus* given by Maupas and Seurat:

Females, 20–22 mm. in length, with a maximum breadth, behind the vulva, of about 0·35 mm.

Head, about 0·035 mm. in diameter. Oesophagus, 0·59 mm. long.

Anus, about 0·1 mm. from the truncated posterior extremity of the body.

Vulva, 9–10·5 mm. from the posterior extremity, *i.e.* situated a short distance behind the middle of the body.

The ovejectors are very long, unequal, both directed backwards.

Eggs, 0·225–0·26 mm. long by 0·09–0·11 mm. broad.

The females of *N. mauritanicus* can be distinguished from those of *N. dromedarii* by the position of the vulva which is located in the posterior body-region in the former species, whilst in the latter it is situated one-third of the body-length from the anterior end.

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EXPLANATION OF LETTERING.

ba. barb-like process of spicule; *gub.* gubernaculum; *int.* intestine; *l.* lip of cloaca; *ov.* ovary; *ovj.* ovejector; *p.* process of body-wall in neighbourhood of vulva; *ut.* uterus.

STRONGYLID PARASITES OF HORSES IN THE PUNJAB.

BY C. L. BOULENGER, M.A., D.Sc.

Professor of Zoology, University of the Punjab, Lahore.

(From the Pathological Laboratory, Punjab Veterinary College.)

(With 5 Text-figures.)

INTRODUCTION.

ALTHOUGH some of the earliest work on the Strongylid parasites of horses was done by Giles in India in 1892, the subject has been almost completely neglected in this country since that date; even the comparatively recent list of Indian parasites compiled by Gaiger (1910) makes no attempt to differentiate the numerous Nematodes which had been confused under the names *Sclerostomum equinum* and *S. tetracanthum* until the appearance of Looss' famous monograph in 1902.

Since the publication of this work the subject has attracted the attention of a large number of helminthologists both in Europe and America, and the sclerostomes of the equines are now referred to eight genera comprising more than fifty species.

Since my arrival in India in October, 1920, I have had the opportunity of examining a large number of parasites of horses both from Lahore and other districts of the Punjab. The work was carried out in the laboratories of the Punjab Veterinary College, the specimens studied there comprising not only those preserved in the college collections, but also large numbers of fresh worms obtained from the dissection and post-mortem rooms.

I am greatly indebted to Lieut.-Colonel G. K. Walker, C.I.E., Principal of the Punjab Veterinary College, for his courtesy in placing the resources of this fine institution at my disposal; my thanks are also due to Mr W. Taylor, I.C.V.D., for much assistance in the laboratory and post-mortem rooms, and to Captain F. M. Stewart, R.A.V.C., Remount Dépôt, Sargodha, for the supply of a number of specimens from that dépôt.

As shown below the worms observed by me are referred to twenty-one species, whilst the list is obviously by no means a complete one I think it can claim to include the majority of species of common occurrence in the Punjab; no new forms were obtained, the present paper however contains additional

information on the structure and development of some of the lesser known species as well as remarks on their geographical distribution.

The majority of the species of horse sclerostomes seem to have a very wide distribution, many forms being now known from five continents, there seem however to be a few (*e.g.* certain species of *Triodontophorus*) with a more restricted range, these are discussed below under the appropriate headings.

Genus STRONGYLUS Mueller, 1780.

(*Sclerostoma* Rudolphi.)

1. *Strongylus equinus* Mueller, 1780.

This species was found in almost every horse examined in the Punjab; from the account given by Looss (1902) and my own observations it appears relatively more abundant in the East than in Europe. I have found *S. equinus* of rather uncommon occurrence in England, and Kotlán (1919) has also called attention to the comparative rarity of the species in Hungary.

2. *Strongylus edentatus* (Looss, 1900).

The second of the larger species of *Strongylus* was found in abundance at Lahore, both in fresh material and among the specimens preserved in the Veterinary College collections.

3. *Strongylus vulgaris* (Looss, 1900).

This, the smallest of the species of *Strongylus*, was also found to be of common occurrence.

Genus OESOPHAGODONTUS Railliet and Henry, 1902.

4. *Oesophagodontus robustus* (Giles, 1892).

Only a single specimen of *O. robustus* occurred among some material labelled *Sclerostoma* sp. in the Punjab Veterinary College collection. The worm was in a much damaged condition, but there is little doubt as to its correct identification.

This species was originally described by Giles from horses and mules in India, I was able to record its occurrence in horses in England (1916) and it has recently been rediscovered in that host in Canada (Ransom and Hadwen, 1918) as well as in a Chapman's zebra from the London Zoological Gardens (Turner, 1920).

Genus TRIODONTOPHORUS Looss, 1902.

Five species of this genus have now been recorded (*cf.* Boulenger, 1916), their geographical distribution presenting certain features of interest.

The type species, *T. serratus*, as well as *T. minor*, were originally described by Looss from Egypt (1902) and until recently were not definitely known to exist elsewhere. I was able to show (1916) that the British representatives of

the genus belong to three quite distinct species, since discovered by Ransom and Hadwen (1918) to be common forms in Canada also. *T. serratus* and *T. minor* were not recorded from the N. American material, nor were they found by me among thousands of specimens examined from horses in England. Our present knowledge of the subject therefore seems to suggest that they are restricted to tropical and subtropical countries.

T. serratus is now known from Egypt (Looss, 1902), Ceylon (? v. Linstow, 1904) and East Africa (Boulenger, 1920). I have also seen undoubted examples of this species obtained from horses in Mesopotamia¹.

T. minor has been recorded from Egypt (Looss, 1902) and West Africa (Yorke and Macfie, 1902 b). I am also able to show in this paper that it is the commonest representative of the genus in the Punjab.

5. *Triodontophorus minor* (Looss, 1900).

As mentioned above this species proved to be the commonest form of *Triodontophorus* in the Punjab, it was found, usually in small numbers, in the majority of horses examined by me in Lahore. As pointed out by Looss the species is remarkable for its habitat in the posterior region of the colon, in the Punjab occurring in this situation usually in company with specimens of *Cylicostomum insigne*.

The Indian specimens of *T. minor* agree on the whole fairly well with those described by Looss from Egypt, there are however a few points to which I desire to call special attention and I therefore add a short description of the worms observed by me.

T. minor is a rather small species of the genus *Triodontophorus*, the Punjab specimens measuring: males, 9–12 mm.; females, 11–14 mm. in length.

The mouth-collar is depressed at the margins (Text-fig. 1, *m.c.*), the latter in some individuals having a tendency to curl forwards. The elements of the leaf-crowns number about 50.

The oral capsule is comparatively large, the breadth being a little greater than the height, as shown by the following measurements: height, 0.13–0.17 mm.; breadth, 0.15–0.185 mm.

The structure of the capsule-teeth varies considerably in different specimens, in some, as in those described by Looss from Egypt, the anterior margins are quite smooth, without denticulations, in others, however, such denticulations occur, a few individuals having the margins of the teeth deeply serrated (Text-fig. 1, *t*). I have shown below that similar variations are found in other species of *Triodontophorus*, the nature of the tooth-margin is therefore not to be depended on as a specific character within the genus.

¹ The worms recorded by Leiper (1910) under this name from horses in London were identified at a time when only two species of the genus were known. It would seem desirable to re-examine this material in view of our present knowledge of the subject before accepting them as belonging to Looss' species.

In the female the distance of the vulva from the posterior extremity is 0.6–0.7 mm. The eggs measure 0.08–0.09 mm. \times 0.04–0.05 mm.

The bursa of the male has a long dorsal lobe; the dermal collar completely surrounds the genital cone, but is better developed on the anterior or ventral surface.

6. *Triodontophorus intermedius* Sweet, 1909.

This species was found on several occasions, both in horses dissected in Lahore and among material sent from Sargodha by Capt. Stewart.

T. intermedius evidently has a world-wide distribution, being now known from Europe (Boulenger, 1916), Asia, E. Africa (Boulenger, 1920), W. Africa (Yorke and Macfie, 1902 *b*), N. America (Ransom and Hadwen, 1918) and Australia (Sweet, 1909).

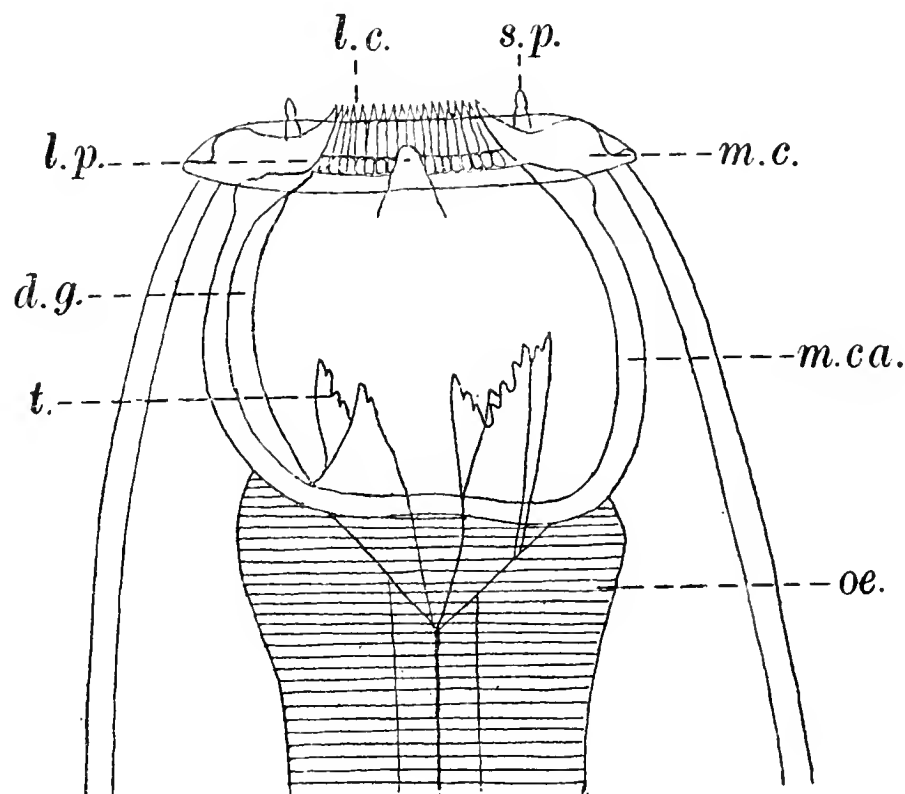


Fig. 1. *Triodontophorus minor* Looss. Lateral view of anterior extremity. \times 200.

Among the specimens observed by me in Lahore were two in which the teeth of the mouth-capsule were without denticulations, having the margins quite smooth as in normal specimens of *T. brevicauda* and *T. minor*. As mentioned above, this character is therefore one of no great systematic importance.

7. *Triodontophorus brevicauda* Boulenger, 1916.

T. brevicauda was obtained on one occasion only, from a horse dissected at the Punjab Veterinary College. Originally discovered in England (Boulenger, 1916) the species has since been recorded from N. America (Ransom and Hadwen, 1918), it obviously has a wide distribution.

Genus POTERIOSTOMUM Quiel, 1920.

(*Hexodontostomum* Ihle, 1920.)

8. *Poteriostomum imparidentatum* Quiel, 1920.

(*Hexodontostomum markusi* Ihle, 1920; *Cylichnostomum zebrae* Turner, 1920.)

A single male specimen of this worm was found in company with numerous examples of *Cylicostomum insigne* in the colon of a horse from Sargodha, Punjab. The species was already known from this host in Europe and in W. Africa (Yorke and Macfie, 1920 *a*), it has also been recorded from a Chapman's zebra in the London Zoological Gardens (Turner, 1920).

The systematic position of the genus has recently been discussed by several authors, I agree with Ihle (1920 *b*) and Yorke and Macfie (1920 *a*) that it should be separated from *Cylicostomum*.

Poteriostomum imparidentatum has been fully described on a number of occasions, there are however a few discrepancies between the accounts given by different authors which, although possibly due to errors of observation, may indicate that more than one species have been confused under this name. To avoid further confusion I have given figures and a short description of the form before me.

The specimen from the Punjab was a male, 11 mm. in length, with a maximum thickness of about 0.6 mm. When fresh the body of the worm was of a bright, blood-red colour.

The mouth-collar is well marked off from the rest of the head and is comparatively low (Text-fig. 2). The mouth is oval in shape, the dorso-ventral axis being somewhat the greater. The sub-median head-papillae are short, with broad bases resting on the oral collar; the lateral papillae do not project.

The elements of the external leaf-crown are small and pointed, numbering about 72. The internal crown consists of 38 leaves, larger than those of the external leaf-crown, of these six (two lateral and four approximately sub-median in position) are considerably longer than the others, reaching almost to the mouth-opening and moreover projecting further inwards than the smaller elements, the latter number six or seven between each pair of long leaves.

In their key to the species of *Poteriostomum*, Yorke and Macfie (1920 *a*) give the number of small elements between the longer ones as seven in *P. imparidentatum*, in their figure however they show only six. Ihle (1920 *b*) gives the number as six, seven or eight, whilst Turner (1920) shows as many as ten in one part of the leaf-crown (p. 447, fig. 2), three only in another (p. 446, fig. 1). The number of leaves in the crown is thus obviously capable of much variation and I agree therefore with Ihle (1920 *b*) in considering *P. pluridentatum* Quiel as merely a variety of the type-species.

The mouth-capsule has a height of about 0.07 mm., the greatest breadth in the dorso-ventral axis measuring 0.2 mm. The walls of the capsule diverge from before backwards and are considerably thickened posteriorly.

In the specimen before me the dorsal gutter is well developed and most conspicuous (Text-fig. 2 A), it extends more than half-way along the dorsal wall of the oral capsule. Both Ihle and Yorke and Macfie describe this organ as present in *P. imparidentatum*, Turner however definitely states that the dorsal gutter is absent and her figure of the dorsal view of the mouth-capsule

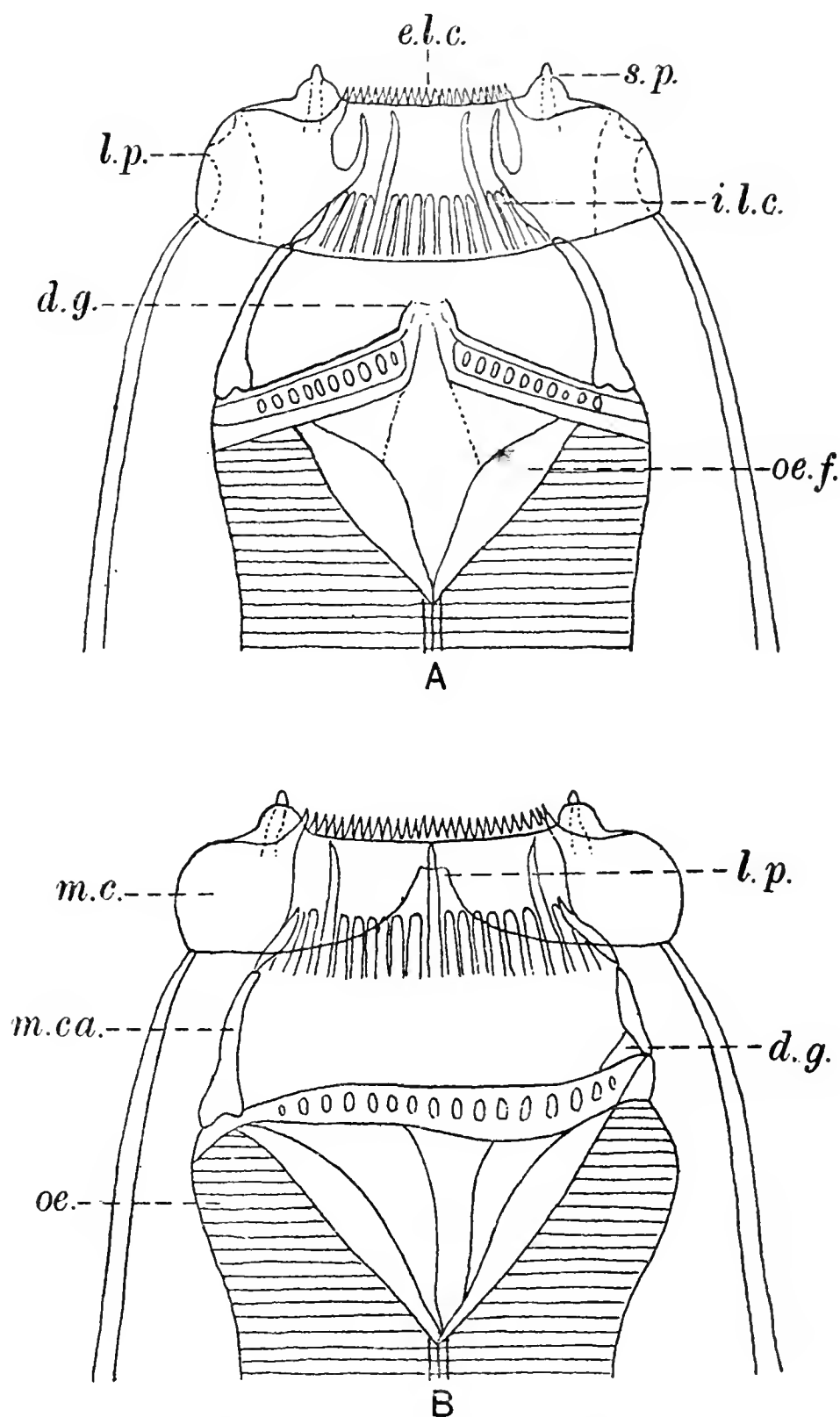


Fig. 2. *Poteriostomum imparidentatum* Quiel. Anterior extremity. A. Dorsal view. B. Lateral view. $\times 200$.

(p. 446, fig. 1) shows no trace of it. If this is not due to some error of observation the specimens described by this author from the zebra must be considered as belonging to a distinct species.

The oesophagus has a length of 0.7 mm., the oesophageal funnel is strongly developed. The cervical papillae and the excretory pore are at approximately the same level, 0.55 mm. from the anterior extremity of the body.

In the structure of the male bursa the Punjab specimen agrees with those described by previous authors. As pointed out by Yorke and Macfie the arrangement of the bursal rays forms the strongest argument in favour of the separation of this worm from *Cylicostomum*.

Genus CYLICOSTOMUM Railliet and Henry, 1902.

(*Cylichnostomum* Looss, 1902.)

9. *Cylicostomum coronatum* (Looss, 1900).

A very common parasite in the Punjab, found on numerous occasions in horses from various sources, sometimes in considerable numbers.

10. *Cylicostomum labiatum* (Looss, 1902).

This species was also obtained on several occasions, usually however in small numbers.

11. *Cylicostomum poculatum* (Looss, 1900).

Found twice in horses from the Sargodha Depôt.

12. *Cylicostomum longibursatum* Yorke and Macfie, 1918.

(*C. calicatifforme* Kotlán, 1919. *C. nanum* Ihle, 1919.)

13. *Cylicostomum minutum* Yorke and Macfie, 1918.

(*C. catinatum* var. *minus* Kotlán, 1920.)

14. *Cylicostomum calicatum* (Looss, 1900).

Species 12–14 were obtained on several occasions from material sent by Capt. Stewart from Sargodha.

15. *Cylicostomum goldi* (Boulenger, 1917).

This species was described by me from horses in England in 1917, it has since been recorded from the same host in Holland (Ihle, 1920 *a*) and in Canada (Ransom and Hadwen, 1918). Miss Turner (1920) tentatively refers to *C. goldi* a single female specimen obtained from a Chapman's zebra in the London Zoological Gardens, at the same time suggesting that this species and *C. pseudo-catinatum* Yorke and Macfie, 1918, may prove to be identical.

It is difficult to understand how the two forms can be confused, they of course belong to the same group of species, the *alveatum-catinatum* group (cf. Ihle, 1920 *b*) the members of which have a number of characters in common, but differ from one another in several important features, particularly in the structure of the oral capsule the walls of which converge from before backwards in *C. pseudo-catinatum* and diverge in *C. goldi*. Another important difference between the two species is to be found in the character of the caudal region of the female which is bent dorsally to a much greater extent in *C. pseudo-catinatum* than in *C. goldi*.

The Punjab specimens of *C. goldi* are a little larger than those observed by me in England, the females reaching a maximum length of 7·8 mm.

My original description of the oesophageal funnel in this species was not quite complete and there was a small inaccuracy in my account of the worm which, whilst not much affecting the relationship between *C. goldi* and *C. pseudo-catinatum*, may have led to confusion in another direction. In my description of the former species the dorsal gutter was stated to be absent, examination of further material however shows this organ to project as a small tubercle on the dorsal side of the base of the mouth-capsule (Text-fig. 3 B)¹.

The oesophageal funnel in *C. goldi* is very well developed and as shown in my original figure (1917, p. 210) is lined by thick chitinous walls; from the latter three thin plates radiate into the funnel, their triangular distal margins frequently projecting into the cavity of the oral capsule so as to give the appearance of small teeth (Text-fig. 3 A).

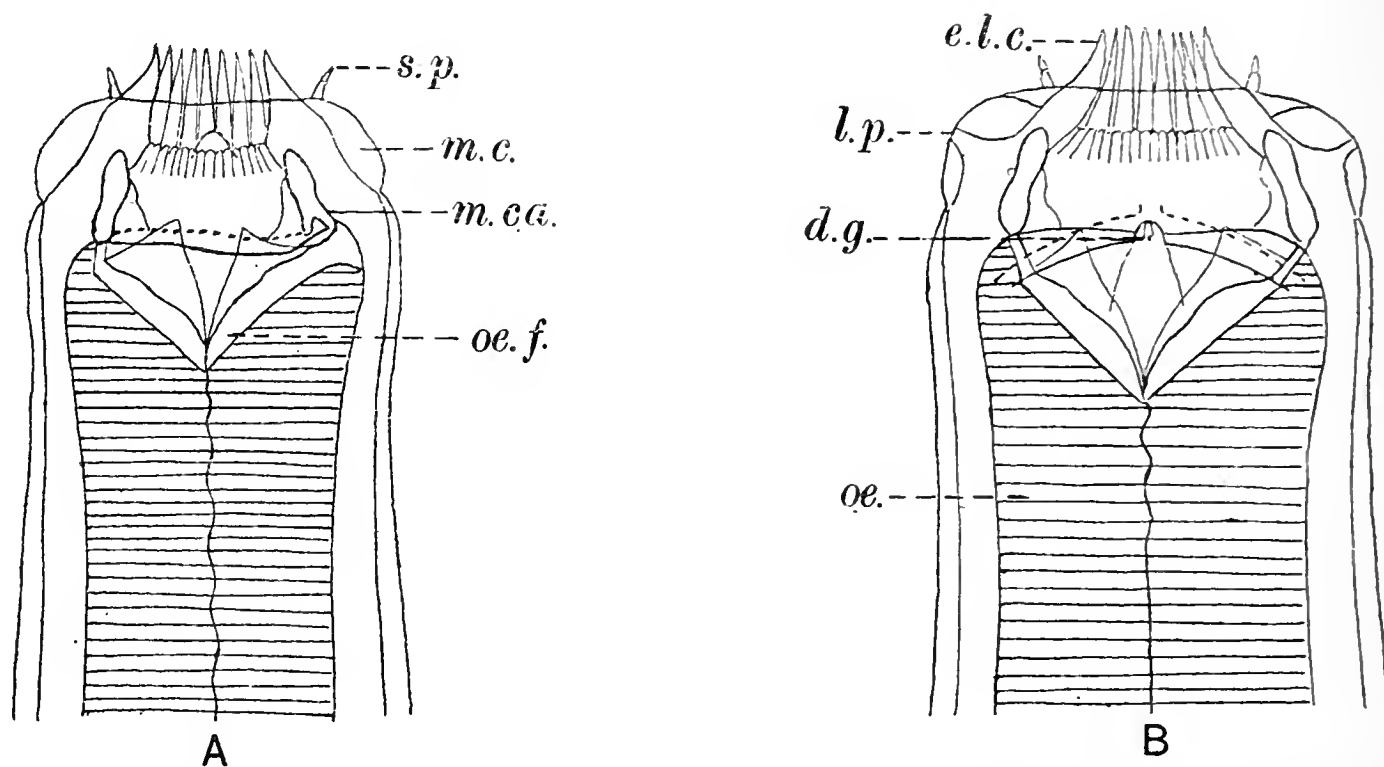


Fig. 3. *Cylicostomum goldi* Boulenger. Anterior extremity. A. Lateral view. B. Dorsal view. $\times 350$.

Yorke and Macfie have recently (1920) described a new species of *Cylicostomum*, *C. tridentatum*, from W. Africa, which, except in the structure of the male genital appendages, seems to agree exactly with my description of *C. goldi*, as modified above. As the genital appendages of certain species seem capable of some variation, a careful comparison of specimens of *C. tridentatum* and *C. goldi* is desirable.

16. *Cylicostomum catinatum* (Looss, 1900).

C. catinatum was found on several occasions, usually in company with *C. pseudo-catinatum*.

17. *Cylicostomum pseudo-catinatum* Yorke and Macfie, 1918.

One of the commonest forms in the Punjab. The genital appendages in this species are liable to a certain amount of variation, individuals being met with

¹ I have to thank Dr J. W. S. Macfie for calling my attention to this error.

in which these organs are provided with three pointed processes instead of the normal two (Text-fig. 4). This character is therefore one which should be used with caution in differentiating the various species of *Cylicostomum*.

18. *Cylicostomum pateratum* Yorke and Macfie, 1919.

(*C. cymatostomum* Kotlán, 1919.)

A single female specimen of this species was found in the colon of a horse from Sargodha, Punjab. The worm was 11 mm. in length, and in its various measurements agreed well with the original examples described by Yorke and Macfie, being distinctly smaller than the Hungarian specimens recorded by Kotlán under the name *C. cymatostomum* and which, according to Ihle (1920 *b*), should be referred to *C. pateratum*. The species has hitherto been recorded from Europe only.

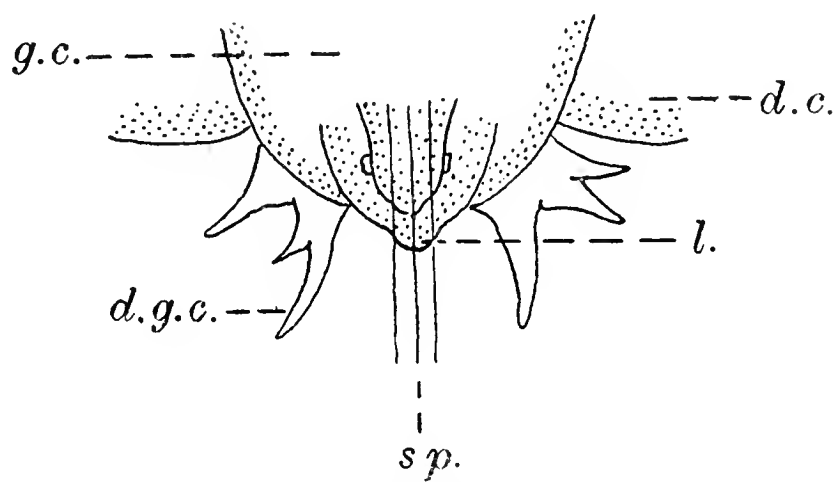


Fig. 4. *Cylicostomum pseudo-catinatum* Yorke and Macfie. Genital appendages of an abnormal male individual, ventral view. $\times 460$.

19. *Cylicostomum nassatum* (Looss, 1900).

20. *Cylicostomum nassatum* var. *parvum* Yorke and Macfie, 1918.

With the exception of *C. insigne*, this is the commonest species of *Cylicostomum* met with in horses in the Punjab. The small variety is more abundantly represented in my collections than the type form, the females of the two varieties are however sometimes difficult to distinguish.

21. *Cylicostomum insigne* (Boulenger, 1917).

C. insigne was the commonest species found in the Punjab, it was obtained from almost every horse dissected in Lahore, frequently occurring in very large numbers. I am able to add somewhat to my account of the species published in 1917.

The distribution of the worm in the body of the host is peculiar: adult specimens were found to be almost completely restricted to the posterior part of the colon, only rarely occurring in the anterior region of this organ; larvae, on the other hand, were found abundantly in the caecum, usually encysted in the sub-mucosa, rarely free, in the latter condition being also occasionally met with in the anterior colon.

The cysts in the wall of the caecum appeared of a bright, blood-red colour, each on being opened was found to contain a cavity, filled with blood, in which the larvae lay coiled. These larvae were also blood-red in colour; they varied in length from 6 mm. to 11 mm., the larger forms being also found free in the cavity of the caecum.

The smallest larvae (6–7 mm. in length) differ markedly in their structure from the adult worms; the characteristic leaf-crowns and the oral capsule are not yet developed, the latter being replaced by a provisional mouth-capsule

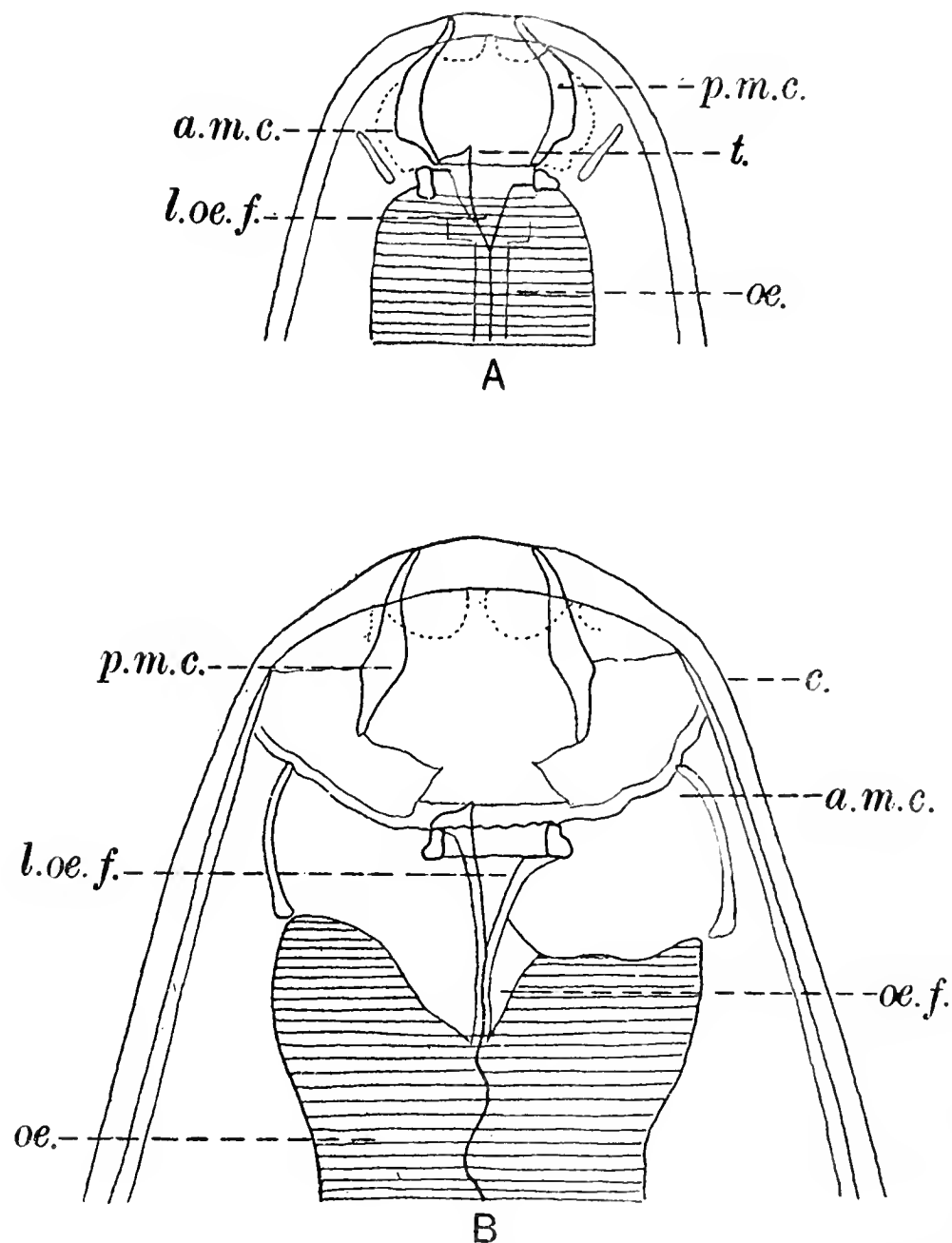


Fig. 5. *Cylicostomum insigne* Boulenger. Lateral views of anterior extremity of larval forms. A. Young larva, 6 mm. in length. B. Older larva, 11 mm. in length. $\times 200$.

of a different shape. The cavity of this capsule is narrowed anteriorly and posteriorly, its walls are thick and in optical section (Text-fig. 5 A) appear to taper at the two extremities. An oesophageal funnel is present from the dorsal side of which a pointed tooth projects into the cavity of the mouth-capsule. This tooth is well adapted for piercing the tissues, there can be little doubt that the larvae when present in large numbers are capable of inflicting serious injury on their hosts.

At this stage the tail of the larva is narrow and pointed, there is as yet no differentiation into the two sexes.

In slightly older larvae a series of cavities can be seen developing behind the provisional mouth-capsule, these are the rudiments of the adult oral capsule (Text-fig. 5), the chitinous walls of which soon also make their appearance. A little later the first signs of an approaching ecdysis are noted, the cuticle of the larva appearing double.

In the largest larvae (11 mm. long) both provisional and adult mouth-capsules are quite distinct (Text-fig. 5 B), the adult oesophageal funnel has also made its appearance and is seen to surround the provisional funnel. At this stage differentiation of the sexes has also begun and in the males the bursa can be distinctly traced, still however enclosed within the cuticle of the pointed larval tail.

When the final ecdysis occurs the larval cuticle is shed, carrying away with it the provisional mouth-capsule together with the funnel and its tooth.

The adult worms met with in the posterior part of the colon are also blood-red, but of not quite so bright a colour as the larvae. I have little to add to my original description except with regard to the structure of the oesophageal funnel.

In preserved specimens, killed by immersion in hot alcohol, the whole body is fixed in an extended condition, in this state the anterior region of the oesophagus appears narrow, with thick muscular walls surrounding a small cavity scarcely sufficiently developed to call an oesophageal funnel (Boulenger, 1917, Fig. 3 a). Observation of the living worms has shown me that during life the anterior division of the oesophagus is capable of expansion and contraction to an extraordinary degree, so that when the muscles of this region are fully contracted its cavity appears correspondingly expanded, forming a large, wide funnel which can be seen to be lined by a delicate membrane, evidently continuous with that covering the inner surface of the mouth-capsule.

In this contracted condition the anterior part of the oesophagus appears very broad and is sharply marked off from the rest of the organ by a narrow "neck." This is the condition met with in the worms, obtained from a zebra in E. Africa, which were described by me under the name *C. zebrae* (1920, Figs. 3 and 4), these now appear to be much contracted specimens of *C. insigne*. I was able to obtain a similar appearance in the latter species by allowing specimens to die in cold, weak alcohol.

C. insigne is now known from Europe, Asia, E. Africa (in the zebra) and N. America (Ransom and Hadwen, 1918).

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EXPLANATION OF LETTERING.

a.g.c., appendage of genital cone; *a.m.c.*, adult mouth-capsule; *c.*, larval cuticle; *d.c.*, dermal collar of genital cone; *d.g.*, dorsal gutter; *e.l.c.*, external leaf-crown; *g.c.*, genital cone; *i.l.c.*, internal leaf-crown; *l.*, ventral lip of genital cone; *l.o.e.f.*, oesophageal funnel of larva; *l.p.*, lateral papilla of head; *m.c.*, mouth-collar; *m.ca.*, mouth-capsule; *oe.*, oesophagus; *oe.f.*, oesophageal funnel; *p.m.c.*, provisional mouth-capsule of larva; *s.p.*, submedian head papilla; *sp.*, spicule; *t.*, tooth.

SOME OBSERVATIONS ON THE BIOLOGY AND STRUCTURE OF *ORNITHODORUS* *MOUBATA*, MURRAY.

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TOGETHER WITH

A NOTE ON THE EXTERNAL CHARACTERS WHICH SERVE TO DIFFERENTIATE THE SEXES.

BY G. H. F. NUTTALL, F.R.S.

(With Pl. XVI and 5 Text-figures.)

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INTRODUCTION.

AT the suggestion of Prof. Nuttall, F.R.S. the following observations were made in the years 1913-14, in continuance of an investigation unavoidably left unfinished by a former student, the late Mr Gordon Merriman¹. The original proposal was to supplement what has already been published, regarding the biology and structure of *O. moubata* [*vide* Nuttall and Warburton (1908), Nuttall (1911 *a* and *b*) and Nuttall and Merriman (1911)] and the observations are presented under these headings in Sections I and II respectively.

The records are incomplete in many ways, because nearly all the living

¹ Some of Merriman's data have been utilised and are fully acknowledged in the text.

material was destroyed by a breakdown of an incubator at Oxford¹ in January 1915, which prevented the confirmation or elucidation of many interesting features in the bionomics of this tick.

SECTION I. BIOLOGY OF *O. MOUBATA*.

Nuttall and Warburton (1908) emphasise the fact that very little information is available regarding the biology of this tick. The number of nymphal stages has not been established and it has been alleged that the imaginal stages may undergo ecdysis. The following observations were made therefore to determine the number of moults undergone by *O. moubata* before and after reaching maturity, the changes taking place in the external anatomy of the ticks at each stage of development, and the duration of the different stages at different temperatures. At the same time records were kept of oviposition, copulation, longevity of the female and engorgement.

Experimental Procedure. These observations were carried out on a series² of isolated females kept at different temperatures. The adult ticks were kept in small glass-topped entomological boxes and their progeny in glass bottles of convenient size, plugged with cotton-wool. Small pieces of filter-paper, placed inside these containers, served to provide hiding places for the ticks and also to absorb the excrement. In the intervals between meals, these receptacles were maintained at constant temperatures in incubators. The ticks were fed upon a fowl, being placed in glass cylinders of small diameter, which could be held against the bare skin beneath the fowl's wing. The individuals, hatched from each batch of eggs, were fed and reared as one group but, to avoid confusion of stages, the newly moulted ticks were separated daily from those which had not moulted. The lighter colour and softer texture of the integument easily distinguished the former from the latter. In some cases the ticks were weighed and measured both before and after feeding and the periods during which they remained attached to the fowl were recorded accurately. The parent ticks used in the following experiments were reared from nymphs received on 3. iii. 1913 from Livingstone, N. Rhodesia (*Quick Lab. Cat. No. 2040*), the stock material being therefore in a healthy condition. When a sufficient number of adult ticks had been reared from this stock, three series of six females were isolated and maintained at 22°, 30° and 37° C. respectively, together with their respective progeny. Newly emerged males

¹ After May 1914, the work was carried on, by permission of Prof. Bourne, in the Zoological Laboratory, University of Oxford.

² Merriman kept records of five ticks only, reared at one temperature, from the first nymphal to adult stages. It was considered that results of greater value would be obtained by commencing with a series of females of known history and by rearing a portion of the progeny of each, at different temperatures. The work became very laborious, owing to the number of individuals involved and their feeding was, in consequence, somewhat irregular, as great care was necessary to avoid confusion of the different batches or individuals.

The results of experiments conducted late in 1912 and early in 1913 were discarded, as the stock material at this date was derived from old females, which had undergone prolonged starvation, and was therefore of poor vitality. The immature stages raised from these females failed to pass beyond the third nymphal stage, probably because of this fact.

were allowed access to the females as they became available and additional males were introduced as required. Both sexes were fed about every 30-40 days, usually when oviposition had temporarily ceased or when, from their general appearance, the females seemed to require food. The presence of the spermatophore in the genital aperture of the female can only be determined by handling the tick, and a few days after copulation it may become loosened and fall away. For this reason, the records of copulation are limited in number because it was considered advisable to disturb the females as little as possible between the oviposition periods.

Experimental Records relating to females kept at different temperatures.

It is necessary, at the present time, to condense experimental evidence for economy of space and therefore the records are presented synoptically below, Synopses I, II and III recording the life-histories of females maintained at 22°, 30° and 37° C. respectively:

Synopsis I. *Females and progeny maintained at 22° C.*

Time reckoned in days from date of emergence of female.

No. of ♀	Date of emergence	Times of feeding	♂ added	Copulation observed	Commencement of oviposition after ♂ added	Oviposition			Death of ♀
						Period of life	No. of eggs	No. of larvae	
26	1. vi. 13	5	30	32	48	78	6	5	862
—	—	89	—	135	—	81	11	11	—
—	—	134	—	—	—	83-85	43	43	—
—	—	172	—	—	—	89	7	7	—
—	—	229	—	—	—	102	11	10	—
—	—	281	—	—	—	105-110	76	70	—
—	—	—	—	—	—	125	5	4	—
—	—	—	—	—	—	172-196	46	45	—
—	—	—	—	—	—	215-220	18	10	—
27	26. vi. 13	15	5	—	195	200-201	26	23	643
—	—	64	—	—	—	220-224	18	8	—
—	—	109	—	—	—	—	—	—	—
—	—	152	—	—	—	—	—	—	—
—	—	230	—	—	—	—	—	—	—
28	23. vi. 13	15	72	72	25	97-98	48	47	730
—	—	72	—	—	—	101-102	24	24	—
—	—	112	—	—	—	104-105	11	11	—
—	—	150	—	—	—	187-195	51	39	—
—	—	227	—	—	—	209-216	115	103	—
29	1. vi. 13	5	30	—	27	57-58	73	71	780
—	—	89	—	—	—	60-64	56	52	—
—	—	134	—	—	—	67	9	8	—
—	—	172	—	—	—	201-231	112	112	—
—	—	232	—	—	—	245-252	49	41	—
—	—	290	—	—	—	—	—	—	—
30	1. vi. 13	5	30	90	16	46-47	57	52	723
—	—	89	—	—	—	51	53	53	—
—	—	134	—	—	—	54-55	11	11	—
—	—	172	—	—	—	59	3	3	—
—	—	228	—	—	—	109-113	125	120	—
—	—	288	—	—	—	115-131	103	99	—
—	—	—	—	—	—	222-236	122	103	—
—	—	—	—	—	—	246-258	61	49	—
31	1. vi. 13	5	30	—	32	62-64	25	23	554
—	—	89	—	—	—	67-68	12	nil	—
—	—	134	—	—	—	71	16	14	—
—	—	178	—	—	—	113-131	39	38	—
—	—	228	—	—	—	222	1	nil	—
—	—	—	—	—	—	252	2	nil	—

Synopsis II. *Females and progeny maintained at 30° C.*

Time reckoned in days from date of emergence of female.

No. of ♀	Date of emergence	Times of feeding	♂ added	Copulation observed	Commence-ment of oviposition after ♂ added	Oviposition			Death of ♀
						Period of life	No. of eggs	No. of larvae	
40	21. vi. 13	13	66	67	13	—	—	—	310
—	—	67	—	109	—	79	5	3	—
—	—	143	—	—	—	81	24	19	—
—	—	206	—	—	—	83	42	27	—
—	—	303	—	—	—	86-90	72	58	—
—	—	—	—	—	—	117-126	175	69	—
—	—	—	—	—	—	150-154	75	70	—
—	—	—	—	—	—	216-223	95	nil	—
41	18. vii. 13	14	39	39	16	55	42	29	279
—	—	40	—	—	—	59	102	88	killed
—	—	82	—	—	—	106	40	nil	by fowl
—	—	117	—	—	—	130	28	20	—
—	—	176	—	—	—	—	—	—	—
42	23. vii. 13	34	42	—	18	60	43	39	385
—	—	42	—	—	—	65	21	16	—
—	—	77	—	—	—	67-68	135	117	—
—	—	112	—	—	—	73	11	7	—
—	—	170	—	—	—	88-92	17	10	—
—	—	271	—	—	—	121-124	88	61	—
—	—	—	—	—	—	186-195	135	79	—
43	23. vii. 13	34	42	277	52	94-100	54	47	481
—	—	42	—	—	—	110	23	16	—
—	—	77	—	—	—	124-126	65	21	—
—	—	114	—	—	—	186-190	54	24	—
—	—	170	—	—	—	—	—	—	—
—	—	273	—	—	—	—	—	—	—
44	23. vi. 13	16	72	—	14	86-87	97	88	415
—	—	72	—	—	—	94	21	9	—
—	—	110	—	—	—	97	16	15	—
—	—	145	—	—	—	125	94	78	—
—	—	201	—	—	—	130-140	81	52	—
—	—	305	—	—	—	314	20	6	—
—	—	—	—	—	—	320	50	nil	—
45	23. vi. 13	16	72	—	16	88-92	12	3	397
—	—	72	—	—	—	94	80	31	—
—	—	107	—	—	—	97-102	111	85	—
—	—	143	—	—	—	115	62	21	—
—	—	200	—	—	—	120-122	64	6	—
—	—	305	—	—	—	155-158	48	22	—

Synopsis III*. *Females and progeny maintained at 37° C.*

Time reckoned in days from date of emergence of females.

No. of ♀	Date of emergence	Times of feeding	♂ added	Copulation observed	Commence-ment of oviposition after ♂ added	Oviposition			Death of ♀
						Period of life	No. of eggs	No. of larvae	
32	26. vi. 13	15	72	81	—	—	—	—	157
—	—	70	—	—	—	—	—	—	—
—	—	154	—	—	—	—	—	—	—
33	21. vi. 13	20	72	—	—	—	—	—	132
—	—	72	—	—	—	—	—	—	—
34	21. vi. 13	20	72	—	9	81	3	nil	118
—	—	72	—	—	—	—	—	—	—
35	21. vi. 13	13	72	89	—	—	—	—	156
—	—	72	—	—	—	—	—	—	—
36	24. vi. 13	15	72	—	—	—	—	—	162
—	—	69	—	—	—	—	—	—	—
—	—	156	—	—	—	—	—	—	—
37	24. vi. 13	15	72	—	—	—	—	—	149
—	—	69	—	—	—	—	—	—	—

* In addition to the above, two other series of six females each, were maintained at 37° C., being started on 13. xi. 1912 and 8. xii. 1913 respectively.

The results were negative except that one female oviposited 57 eggs 29-33 days after emergence, from which no larvae were obtained.

Feeding. In each of the foregoing series of experiments, the female ticks were offered food after the times indicated in the synopses, but they either refused to gorge or took very insignificant quantities of blood. However it is probable, had they been in permanent association with the host and able to select their own times of feeding, that they would have fed over longer periods. In the case of the ticks kept at 37° C., the high temperature adversely affected their vitality and after a period of about 70–100 days they appeared to be unable to feed. The two females, which did feed slightly about the 150th day, died almost immediately afterwards¹.

Copulation usually occurs very shortly after the ticks have finished feeding. In the series of experiments which was discarded, copulation was observed on two occasions when the females were 289 days old and was succeeded in one case by the deposition of fertile eggs after 310 days. Females may be fertilised before engorgement, but no case of fertilisation by an unfed male was noted, probably owing to its non-activity in this state. During the course of these experiments it was found that copulation between individuals of the two species *O. moubata* and *O. savignyi*² could occur and as the females are apparently non-parthenogenetic, it would seem that the stimulus of coition is sufficient to induce oviposition.

No evidence of parthenogenesis. It is of interest to note the regularity with which oviposition commenced about 15 days after the male was allowed access to the female (at 30° C.), irrespective of the age of the female. In each case, the males were newly emerged, fully fed and very active, with the result that the females were probably immediately fecundated. At a temperature of 22° C., the period between the date of introduction of the male and oviposition was more variable, due probably to varying dates of fecundation. At this temperature the activity of the individual was considerably diminished.

To determine whether *O. moubata* could reproduce parthenogenetically, ten females, emerging in June 1913, were reared separately. They were fed in June, July, September and November 1913, maintained at 30° C. between feeds and they died in February and March 1914, without oviposition having occurred, except in one case, in which the female deposited 183 fertile eggs in 15–36 days after emergence on 1. vi. 13. This female was first fed on 6. vi. 13.

It is probable that this female was fertilised unobserved, perhaps while feeding, and further evidence is necessary before parthenogenesis can be considered to be even of rare occurrence in this species.

¹ Some records of the duration and extent of engorgement are given on pp. 336, 337.

² Copulation between individuals of the two species *O. moubata* and *O. savignyi*, when maintained at 30° C.

(a) Two female *O. moubata* were associated with four male *O. savignyi* and fed on 21. v. 13, 25. vi. 13 and 28. viii. 13. A spermatophore was observed attached to the sexual aperture of one female on 27. viii. 13. No oviposition occurred.

(b) Conversely, two female *O. savignyi* and two male *O. moubata* were associated and fed as above. Spermatophores were observed attached to one female on 17. vi. 13 and to the second on 8. vii. 13. Oviposition occurred on 15–16. vii. 13 (32 eggs) and on 11–19. ix. 13 (107 eggs), none of the eggs being fertile.

Oviposition.

There are very few records of this phase of the life-history. The observations of other authors, collated by Nuttall and Warburton (1908), indicate that the eggs are deposited in batches, being agglutinated in masses, at intervals of a few days. At a temperature of 29° C., in a dry atmosphere, one female deposited 17, 51 and 26 eggs at intervals of 3-8 days (Newstead); the number of eggs increased when the female took a large meal, but the maximum number oviposited by one individual was 139 (Dutton and Todd); a fertilised female did not oviposit until after it had fed (Wellman). Herms (1916), on the other hand, has recorded that the female of an allied species, *O. coriaceus* Koch, laid 428 eggs during her first oviposition period.

The data, with reference to oviposition, obtained from the present experiments, are summarised in the following table, minimum, maximum and mean results being shown:

	Ticks kept at					
	22° C.			30° C.		
	Min.	Max.	Mean	Min.	Max.	Mean
No. of days before oviposition occurred after ♂ was allowed access to ♀	16 (aberrant case 195)	48	29	13 (aberrant case 52)	18	15
No. of eggs deposited after each feed (when oviposition occurred)	1	228	90	28	318	111
No. of days between dates of feeding and oviposition commencing or recommencing	0	50	26	7	24	13
No. of days over which oviposition extended after each feed	1	48	18	1	47	10
No. of eggs deposited by one ♀	44	535	240	108	488	350
Percentage of fertile eggs		91 %			58 %	

It will be remembered that all these ticks originated from the same stock, therefore the data obtained from the experiments conducted at the different temperatures are strictly comparable.

When the ticks were maintained at 30° C. oviposition did not occur generally after feeding until a period of a fortnight had elapsed and then it only lasted on the average for 10 days, these periods being increased to twice the length when the temperature was lowered to 22° C. The number of eggs deposited after each feed varied considerably, at 30° C. the minimum, maximum and mean numbers being 28, 318 and 111 respectively, while at the lower temperature (22° C.) the mean figure was as high as 90. The heightened temperature increased by 50 per cent. the average number of eggs (240 at 22° C.) deposited by one female but at the same time the fertility of these eggs was decreased from 91 per cent. to 58 per cent. Approximately equal numbers of larvae were produced therefore in the two series, although of course they were produced in a shorter time at the higher temperature. In these experiments, oviposition ceased when the female approached an age of 250 days, but

it is considered that more careful feeding would probably have increased both the total oviposition period and the number of eggs deposited. Although at these temperatures the maximum number of days over which oviposition extended after each feed was 48, several cases were noted in which females kept at 30° C. produced fertile eggs about 75 days after engorgement. The largest number of eggs produced by one female was 535, of which 91·5 per cent. were fertile, the tick in this case having been kept at 22° C. It is of interest to note that in the incubators, oviposition occurred usually at night, which is in conformity with the natural habit of the tick.

Under laboratory conditions therefore, the influence of the temperature factor on oviposition is very marked, a rise of 8° C. (from 22° C.) practically doubling the rapidity of egg-production after each meal, but at the same time reducing the fertility of the eggs by 30 per cent. A further increase of temperature to 37° C. under these conditions inhibited reproduction almost entirely.

Experiments devised to determine the influence of food on oviposition and fertility were not completed owing to the breakdown of the incubator.

Longevity of females, males and nymphs.

Females. The longevity of the female tick under varying conditions is recorded in the following table, being reckoned in days from the date of moult:

Condition	Temp. ° C.	Longevity in days			No. of individuals observed
		Min.	Max.	Mean	
Performing normal functions	22	554	862	715	6
„ „ „	30	310	481	397	5
„ „ „	37	118	162	145	6
Unfed	37	32	105	78	12

Males: (a) Unfed and kept at 30° C.:

13 males (progeny of ♀ 45), which emerged on 11. xii. 13, lived for minimum, maximum and mean periods of 130, 244 and 184 days.

(b) Allowed one feed and kept at 37° C.:

11 males (progeny of ♀ 41), which emerged on 10. xii. 13 were fed on 15. xii. 13. They lived for minimum, maximum and mean periods of 98, 136 and 123 days.

First-stage nymphs—unfed:

Temp. in ° C.	Date of emergence	Longevity in days			No. of individuals observed
		Min.	Max.	Mean	
22	30. x. 13	305	400	362	23
	24. vii. 13	245	302	267	26
	12. x. 13	310	418	347	110
30	30. x. 13	141	210	179	23
37	30. x. 13	45	62	51	23

The longevity of individuals of either sex is greatly influenced by temperature, the length of life of a normal female, kept at 37° C. being approximately one-fifth that of a similar female kept at 22° C. An unfed female has about half the span of life of a fed female, when both are maintained at 37° C. The series of females kept at 22° C. survived unfed for a mean period of 441 days after ovipositing, whereas at 30° C. the females died off in a quarter of the time.

The male individuals are very resistant to starvation, having an average life of 184 days when kept at 30° C. Unfed first stage nymphs may survive for a year at low temperatures, but they succumb fairly quickly, at 37° C.

No evidence of adult ticks moulting.

According to Nuttall and Warburton (1908) two authors have stated that *O. moubata* moults after reaching maturity: (a) the female tick may moult after oviposition (Dönitz) and (b) the male individuals alone may continue to moult (Möllers).

During these experiments however, although sexually mature ticks were kept alive for long periods, no individual, male or female, was observed to undergo ecdysis, once that individual had reached the adult state.

Duration and number of the nymphal stages at 22° and 30° C.

(a) Experimental Data.

A portion of the progeny of each female was reared separately to the adult stage, to determine the duration of the various nymphal stages and to establish the number of these stages. The individuals, which failed either to feed or to moult after feeding (through not procuring sufficient food or other cause), were rejected at each stage.

It is not considered necessary to present the data in detail for each batch of individuals reared and the records are summarised in Synopsis IV. The minimum, maximum and mean durations of the stages are given, together with the numbers of the individuals on which the observations were made. The larva passes into the first nymphal stage without previous engorgement, but not so the succeeding stages, and therefore it should be noted that after the first nymphal stage the periods required for ecdysis are reckoned from the date of the previous meal and not that of the previous moult. In the majority of cases, the newly emerged individuals were offered a meal as soon as they appeared to be capable of feeding.

Synopsis IV. *Duration of stages in days.*

Stage	Ticks kept at 22° C.				Ticks kept at 30° C.			
	Min.	Max.	Mean	No. of individuals observed	Min.	Max.	Mean	No. of individuals observed
Egg to larva	11	25	16	226	8	13	10	166
Egg to 1st ☉	20	38	23	221	13	19	14	112
1st to 2nd ☉	9	20	12	379	5	8	6	405
2nd to 3rd ☉	9	26	14	303	5	10	8	371
3rd to 4th } ☉	9	22	15	215	6	11	8	274
☉ or adult } ♂	12	18	15	33	7	10	8	16
4th to 5th } ☉	8	35	23	109	7	11	9	128
☉ or adult } ♂	11	35	23	73	8	16	10	93
	♀	10	20	62	8	11	9	35
5th to 6th } ☉	13	—	13	1	9	13	12	7
☉ or adult } ♂	15	21	17	7	9	17	12	13
	♀	25	16	32	7	16	10	77
6th to 7th } ☉	No records				23	—	23	2
☉ or adult } ♀					10	23	15	5

(b) Discussion of Data.

The casualties¹ from one cause or another were very large, as only 36·3 per cent. of a total number of 1233 eggs were reared to the adult stage. About 40 per cent. reached maturity at 22° C. and 33 per cent. at 30° C.

The ratio of the sexes was practically equal, there being 235 males to 212 females among the total number of adults reared.

The number of ecdyses undergone by an individual before reaching maturity varied as shown in the following table:

Number of ecdyses	Percentage of males	Percentage of females
4	20·8	nil
5	70·7	45·7
6	8·5	51·5
7	nil	2·8

As two seventh stage nymphs were obtained but failed to mature, it would appear that occasionally a female individual may undergo eight ecdyses. Males appeared after four to six and females after five to seven ecdyses, but whereas the majority of the males appeared after the fifth, the females appeared in approximately equal numbers after the fifth and sixth ecdyses.

Herms (1916) states that in the allied species, *O. coriaceus* Koch, the number of moults varies from four to seven. He also found, in the case of this species, that metamorphosis occupied a period of at least 159 days (? temperature) which is very considerably longer than was required under the conditions of the foregoing experiments. In the latter at 30° C., only the two last changes, namely the fifth to sixth and sixth to seventh nymphs or

¹ An unrecorded number of individuals of low vitality were rejected because they either failed to moult or refused to feed. Many of these could, no doubt, have been reared to maturity by being fed repeatedly, and would have matured under natural conditions.

equivalent adult stages, occupied more than ten days and a decrease in temperature of 8° C. did not double these periods. In Nature, an individual would require certain periods of quiescence to prepare and recover from ecdysis and very varying periods to enable it to find a suitable host. Ignoring these latter periods, the data given in Synopsis IV indicate that the mean minimum periods required for the metamorphosis of an individual from egg to adult are as follows:

At 22° C.:	♂	64, 87 or 104 days.	
	♀	84 or 103	„
At 30° C.:	♂	36, 46 or 57	„
	♀	45, 55 or 72	„

according to whether the male undergoes four, five or six and the female five, six or seven moults. Therefore an increase in temperature of 8° C. (from 22° C.) approximately halves the average time required for metamorphosis, and at the higher temperature there is more regularity in the individual life-histories. The adults emerge from the previous nymphal stage in the same time as the nymphs of the equivalent stage.

The period of starvation appears to make but little difference in the time required after engorgement for ecdysis, provided of course it is not too prolonged. Two batches of second stage nymphs, in one case, were starved for 6 and 36 days respectively, yet the third stages emerged in mean periods of 11 and 10 days; in another case, third stage nymphs starved for 5 and 18 days moulted in mean periods of 10 and 12 days; in a third case, fifth stage nymphs starved for 9 and 37 days produced females in 14 days.

Preparatory to the emergence of a new stage, the nymphal skin normally splits laterally, as far as the ventro-anterior edge of the spiracle. The slit may not extend to the spiracle or it may go considerably beyond, a long series of specimens showing every variation. In all cases, however, the slit passes or tends to pass immediately under the ventral edge of the spiracle.

The photographs¹ in Plate XVI show the successive stages of the emergence of the first nymph from the larval stage. In the first the larva is still partly enclosed in the egg-shell, in the second it is quiescent, with its legs contracted, preparatory to ecdysis. The first nymph then emerges, leaving its larval skin enclosed within the egg-shell, clearly shown in the last four photographs. The difference in texture between the larval skin and the egg-shell is very apparent. In many cases, however, the larva frees itself from the shell before undergoing ecdysis, as in the case of *O. savignyi*.

Duration and extent of engorgement at each stage.

Engorgement can take place at any stage of the life-history, after the larval stage has been passed, very shortly after emergence, probably as soon as the chitin of the mouthparts has hardened sufficiently to ensure penetra-

¹ These photographs were taken by the late Mr G. Merriman, from balsam preparations.

tion of the host's skin. During these experiments, taking the nymphal stages 1 to 6 in order, the minimum number of days elapsing between emergence and feeding were 5, 2, 2, 1, 3 and 7 respectively, but no special attempt was made to induce early feeding in any case.

Successful ecdysis seems to be dependent on the extent of the previous meal and in the case of *O. moubata* never occurs prior to engorgement, at any stage of the life-history (excepting larva to first nymph). Herms' (1916) statement therefore, that the first stage nymph of *O. coriaceus* Koch moulted to the second stage nymph, without previously feeding, if confirmed, would be of interest.

The two points for consideration in the gorging of ticks are (1) the time of attachment to the host and (2) the quantity of blood they are capable of absorbing. Merriman kept four ticks under observation from the first nymphal stage until they reached maturity and recorded their weights before and after engorgement and the times of attachment to the host. His records showed that the time of attachment was approximately the same with all stages; taken collectively, the minimum, maximum and mean times were 25, 74 and 48 minutes respectively, at room temperature, about 16° C. The period of starvation had no influence on the time required for engorgement nor was the feeding period correlated with the amount of blood absorbed.

The mean increases in weight in grams were 0.0011, 0.0040, 0.0084 and 0.0361 for the first four nymphal stages. The two fifth stage nymphs, which gave rise to females, showed a mean increase in weight of 0.0528 gram and after reaching the adult state of 0.1349 gram. The two males which emerged from the fourth nymphal stage showed a mean increase of 0.0076 gram only.

Merriman therefore concluded that the female fed chiefly after reaching maturity, for the purpose of egg maturation and that the male required most food in the last nymphal stage for the production of spermatozoa. To test this conclusion, ten nymphs were reared separately from June 1913 onwards, Merriman's observations being repeated, but in this case the ticks were maintained at 30° C. between meals. Six individuals were female and four male. In the female series, the minimum, maximum and mean increases in weight in grams were (a) before the final moult, 0.0158, 0.0432 and 0.0272 and (b) after the moult 0.0062, 0.1310 and 0.0492; the corresponding figures for the male series were (a) 0.0158, 0.0164, 0.0122 and (b) 0.0066, 0.0296, 0.0166 grams respectively. For the ten ticks, the period required for moulting varied between eight to ten days only, although the quantities of blood absorbed varied very considerably.

It would appear therefore that the adult of either sex may take its largest meal either before or after reaching maturity, the extent of the meal probably depending on the vitality of the individual. This view is supported by the time records. These show that the ticks which fed heavily secured a sufficiency in short periods of time, e.g. one female gained 0.1012 gram in 23

minutes while a second only gained 0.0344 gram in 65 minutes. Whatever the extent of the meal¹ however, the period required for moulting was unaffected.

The influence of moisture on vitality and ecdysis.

O. moubata is said to prefer dryness. To determine the influence of moisture on metamorphosis, a lot of 150 first stage nymphs from the same female was divided into three, these batches being confined in covered jars and kept at 30° C. between meals. The atmosphere in the first jar was maintained in a saturated condition by the presence of excess of water, to the second jar a very little moisture was added each morning and the air in the third jar was dried by means of a layer of calcium chloride. The first stage nymphs were fed on 18. ii. 1913 and each batch of the succeeding stages on 16. iv. 13, 23. v. 13, 13. vi. 13 and 10. vii. 13 respectively. In the following table the minimum and maximum numbers of days required for ecdysis are given, with the mean in brackets and the number of individuals observed in square brackets:

Atmosphere	2nd ☉	3rd ☉	4th ☉	5th ☉	6th ☉
Saturated	6—8 (6) [28]	6—15 (8) [22]	11 [1]	—	—
Moist	5—8 (6) [40]	6—8 (7) [31]	8—11 (8) [20] 3 ♂	10 [6] 2♂, 3♀, 1 ☉	—
Dry	5—6 (6) [43]	6—8 (7) [36]	6—16 (7) [36] 3♂	7—14 (9) [32] 10♂, 14♀	10—14 (12) [6] ♀

From these records it will be seen that an excess of moisture has no inhibitory influence on ecdysis (cp. Synopsis IV), but that it is decidedly unfavourable to the vitality of the individual, only one tick passing the third nymphal stage under these conditions. Even under medium conditions, the mortality was large only eight ticks maturing, whereas under dry conditions 66 per cent. of the nymphs completed metamorphosis.

This result is of particular interest in relation to some observations of Rodhain (1919), on the distribution of *O. moubata* in tropical Africa. This author considers that the absence of the tick in the lower equatorial regions, in the very humid areas, may be partly, if not entirely, explained by the assumption that these ticks succumb rapidly, in a habitat of this character. Man, the normal host, may certainly be temporarily absent, but occasional hosts, such as warthogs, are frequently abundant.

SECTION II. STRUCTURE OF *O. MOUBATA*.

This section comprises notes on the dimensions of the egg and unfed specimens of the different stages, the changes in form undergone during development by structures of diagnostic value, namely the hypostome, the fourth tarsus and the spiracle, and on the genital apertures of the two sexes.

¹ Forcible detachment, after partial engorgement, would no doubt affect the length of this period, or even prevent ecdysis if it occurred too early.

Dimensions of the egg.

According to Nuttall and Warburton (1908), Dutton and Todd record the size of the egg as 0.9×0.8 mm. During the course of these experiments however, 193 eggs were measured immediately after deposition, being oviposited by four different females. The minimum, maximum and mean measurements of length \times breadth were 0.9×0.8 mm., 1.5×1.1 mm. and 1.06×0.92 mm. respectively. The maximum records approximate closely to figures obtained for the egg of *O. savignyi*. The eggs (mean measurements 1.19×1.0 mm.) of one female were distinctly larger than those of the other three, although their fertility was very low, only 11 per cent. producing larvae. The variation in size in the other batches was negligible even from different parents. The age of the female appears to have very little influence on the size of the eggs, very similar mean measurements being obtained from batches of eggs deposited after intervals of 70 and 140 days.

When first oviposited the egg is light straw-coloured and translucent. It darkens in a few hours at 30° C. but much more slowly at 22° C., at which temperature it may take from 24 to 30 hours to attain the final dark brown colour. The normal coloration is lighter than in the case of *O. savignyi*.

Dimensions of unfed stages.

As very little information is available¹ with regard to the sizes of the different stages, particularly of unfed individuals, and as a considerable amount of material, from one stock, had been bred under control, it was considered that the measurements detailed in Synopsis V would be of interest. These data were obtained by outlining, under the binocular microscope, the dorsal surfaces of *unfed* specimens, the breadth being taken as the distance between the spiracular depressions.

Synopsis V. *Dimensions of stages.*

Measurements in mm. to nearest tenth from unfed specimens preserved in 70 % spirit shortly after emergence.

Stage	Min. Length \times Breadth	Max. Length \times Breadth	Mean Length \times Breadth	No. of individuals observed
Larva	0.8×0.7	1.3×1.1	1.1×0.9	80
1st \odot	1.0×0.7	1.3×1.0	1.2×0.9	74
2nd \odot	1.2×1.0	2.2×1.6	1.6×1.3	54
3rd \odot	1.8×1.4	3.4×2.4	2.7×2.0	23
4th \odot	2.8×2.0	4.5×3.1	3.7×2.5	26
♂ from 3rd \odot	3.8×2.4	4.9×3.3	4.6×3.0	35
5th \odot	3.1×2.2	6.1×4.3	4.6×3.2	39
♂ from 4th \odot	4.0×2.6	6.3×4.3	4.9×3.2	137
♀ from 4th \odot	4.9×3.2	8.1×5.4	6.1×4.0	69
6th \odot	5.3×3.4	—	5.3×3.4	1
♂ from 5th \odot	4.6×3.0	6.0×4.4	5.3×3.5	19
♀ from 5th \odot	5.0×3.2	8.9×5.9	6.6×4.4	78
7th \odot	6.2×3.8	6.9×4.3	6.5×4.0	2
♀ from 6th \odot	6.7×4.3	8.4×5.3	7.4×4.7	(slightly fed) 6

¹ Vide Nuttall and Warburton (1908). It should be noted that prolonged starvation will accentuate the dorso-ventral flattening, therefore the measurements of a tick on emergence and after starvation may be different, the latter figures being the greater.

The dimensions of individuals of maximum size in any one stage exceed those of individuals of minimum size in the succeeding stages. As would be expected, the adults emerging from one nymphal stage are smaller in size (mean measurements) than the adults emerging from the succeeding nymphal stages, presumably owing to the fact that the latter have secured additional food and have had longer growth periods. In addition, considering the fourth to the seventh nymphal stages, the males of any one stage are only slightly larger, whereas the females are considerably larger than the nymphs of the equivalent stage. Partially gorged individuals may require a greater number of meals than normal individuals before completing their metamorphoses, that is to say, the individuals of minimum size have the minimum vitality, are comparatively poor feeders and may require to undergo more moults before reaching maturity. How far, if at all, the extent of engorgement determines the number of nymphal stages still requires elucidation.

Considering the larval and nymphal stages in order, the percentage increases in length are 9.1, 33.3, 68.8, 37.1, 24.3, 15.2 and 22.6 respectively. The 9 per cent. increase from larval to first nymphal stage is brought about by the change in the body contour and not by growth. The highly convex body of the larval stage is flattened dorso-ventrally in the first nymphal stage. The greatest growth in these experiments occurred in the second nymphal stage, the 68.8 per cent. increase in length being accompanied by a 53.9 per cent. increase in breadth, neither of these figures being reached in any of the other nymphal stages. The temperature at which the individuals were maintained between times of feeding had no influence on their growth, the mean figures obtained from material bred at 22° C. and 30° C. varying only by small fractions of a millimetre at each nymphal and equivalent adult stage.

Changes in external anatomy undergone during development.

(a) The Hypostome. Fig. 2.

The changes in the dentition of the hypostome during the development from larva to adult are indicated in Fig. 2, (a) to (h) representing the larval and nymphal organs arranged in order of development, (i) and (j), the hypostomes of females emerging from fourth and sixth nymphs and (k) and (l), of males from third and fifth nymphs respectively.

The teeth are arranged in transverse rows and longitudinal files¹. The number of teeth borne by the hypostome increases each time the tick undergoes ecdysis, both by the addition of teeth in the rows and by the formation of additional files. In the *larva* there are only two rounded teeth on each half of the distal extremity of the hypostome (1/1) their form indicating that they could scarcely serve for attaching the tick to a host. It is well known that the larva moults to the first nymphal stage without previous engorgement. The *first-stage nymph* is, however, an active feeder and the dentition of its

¹ The signs 2/2, 3/3 used hereafter indicate the number of files on each side of the median line.

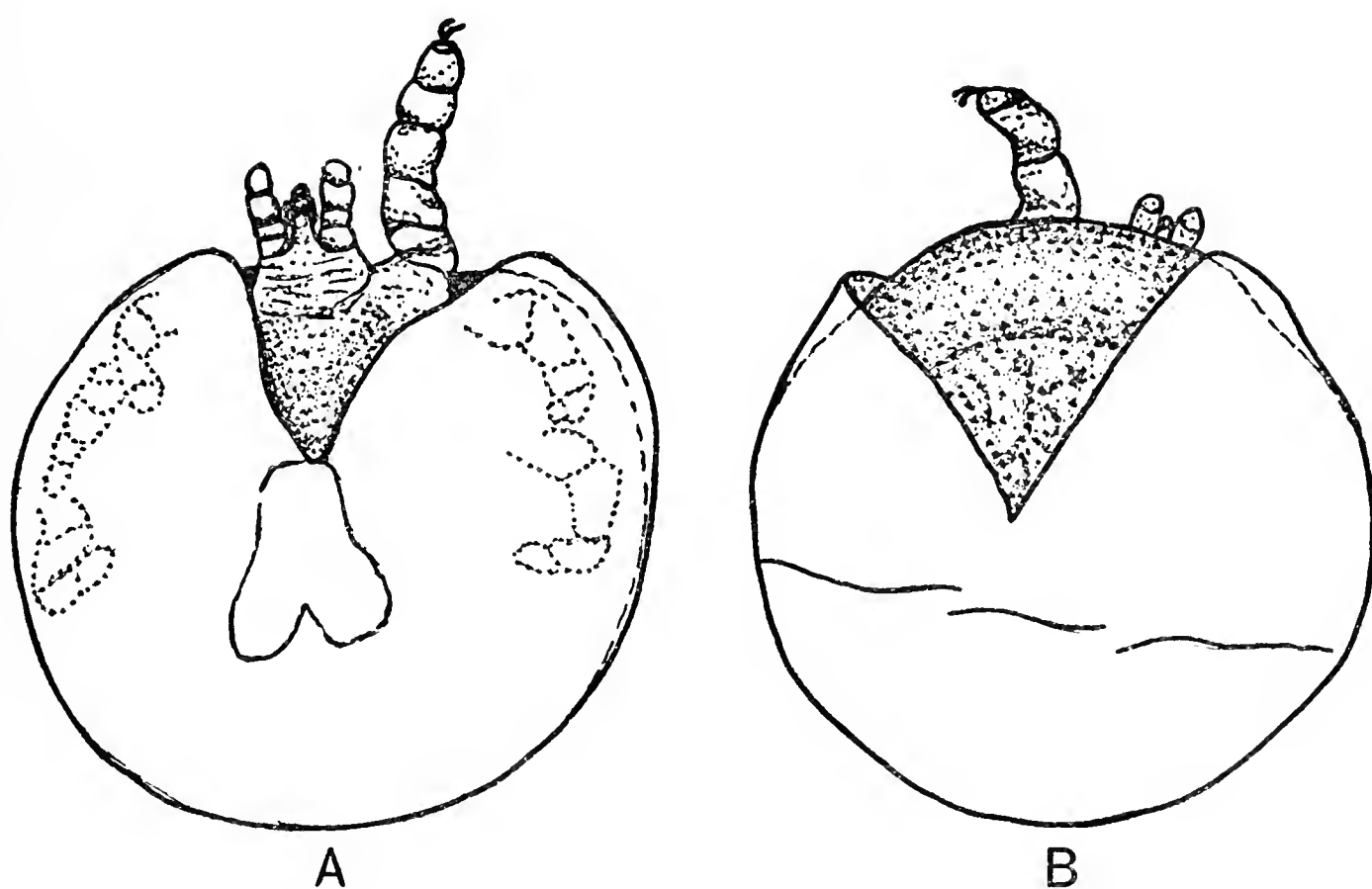


Fig. 1. Showing the larva of *Ornithodoros moubata* protruding from the ruptured egg-shell. (A) dorsal aspect, showing one leg and mouth parts, (B) ventral aspect of same specimen. $\times 65$ (G. M. del. 1912).

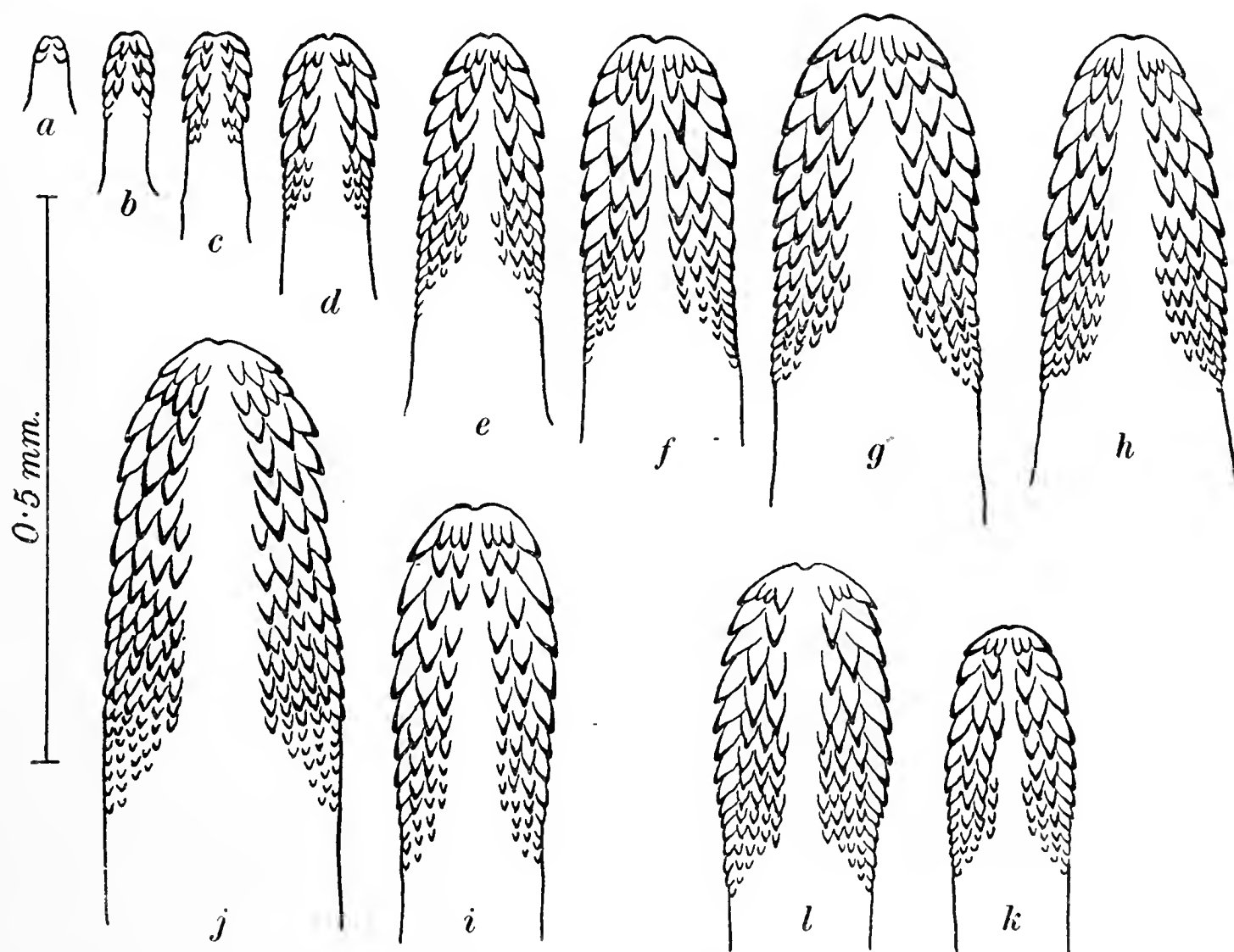


Fig. 2. *Ornithodoros moubata*, hypostomes in ventral aspect: (a) of larva, (b) to (h) of 1st to 7th stage nymphs; (i) and (j) of females, from 4th and 6th stage nymphs; (k) and (l) of males from 3rd and 5th stage nymphs. (N. C. del.)

hypostome is proportionately developed, some of the teeth being well-developed and pointed. They are arranged in five transverse rows, the distal three 2/2 followed by two 1/1, only the former being functional. In the succeeding stages, the hypostome increases in size and the dentition becomes more complex. At the extremities of the hypostome, the teeth are always poorly developed, this being the case at the proximal end particularly, where it is difficult to determine the exact number and arrangement. The hypostome of the *second-stage nymph* shows six transverse rows, of which four are arranged 2/2, a third file commencing at the fifth row. Rows 5 and 6 are but poorly developed.

In the *third-* and *fourth-stage nymphs* the transverse rows number about 8 and 12 respectively, but in the former the functional teeth are still arranged 2/2 while in the latter a third file becomes well-developed at row 4; in addition a fourth file commences at row 6. In the later stages the smaller distal teeth are arranged in two rows. In the *fifth-stage nymph* only row 3 remains 2/2, followed by three rows 3/3 and one or more functional rows 4/4. In the *sixth-* and *seventh-stage nymphs*, the distal teeth are arranged 3/3 for four rows, 4/4 for two rows, and then 5/5 for three or more rows, the remaining teeth dwindling away posteriorly.

The dentition of the *adult* approximates to that of the equivalent nymphal stage, the smaller individuals having the lesser number of files and rows of functional teeth.

Commencing with the larva and taking the nymphal stages in order, the total numbers of teeth, large and small, borne by the hypostomes of the particular individuals to which Fig. 2 relates are 4, 16, 26, 40, 70, 86, 102 and 104¹. Males from third and fifth nymphs show 76 and 102 teeth and females from fourth and sixth nymphs 78 and 160 teeth respectively.

(b) The Legs. Fig. 3.

In Fig. 3 are illustrated the terminal portions of the fourth legs of ticks during the successive stages of development, namely the first to seventh nymphal stages (normal individuals) and the second to fifth nymphal stages (small individuals); of males from the third and fifth nymphs and of females from the fourth and sixth nymphs; and also the tarsi of the first legs of first and second nymphs. The larval leg is illustrated in Fig. 1. In the latter the leg is undeveloped and useless for locomotion, the chitin being exceedingly thin and the claws weak².

The characteristic and diagnostic dorsal humps seen on the protarsus and tarsus of the adult are reduced or absent in the immature stages.

The protarsus of the first nymph carries a slight protrusion which becomes more developed at each successive stage, both normal and small, until in the sixth and seventh nymphal stages, the hump is as prominent as it is in the

¹ This hypostome may not be typical, as only two individuals were available for examination.

² The larva, like the larva of *O. savignyi*, is able to free itself from the egg-shell in many cases.

adult stages. The basal tarsal hump is absent in the first stage, develops as a slight protrusion in the second and gradually assumes a hump-like form in the subsequent stages. But in small individuals of the second and third stages the protrusion is still slight, and as in the first stage there is no trace of the distal hump. In normal individuals however, the latter hump is represented in the second stage by a scarcely perceptible prominence, in the third by a slight protrusion and in the fourth onwards by a definite hump.

The size of the humps is less in the smaller specimens of these later nymphal stages. In the adult all the humps are very marked but the structure of the leg is subject to variation in individual ticks. The distal humps are somewhat

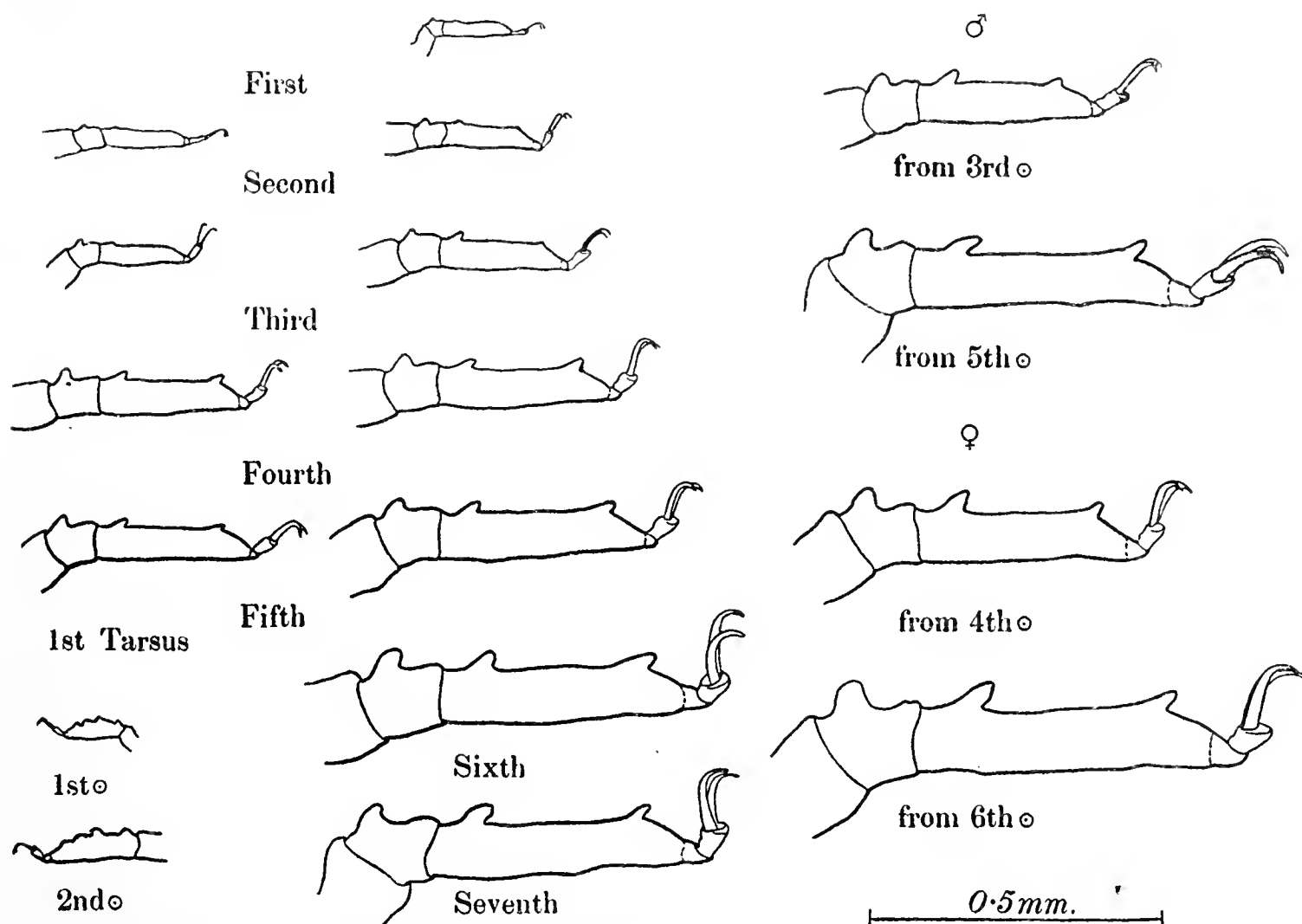


Fig. 3. *Ornithodoros moubata*, tarsi of fourth legs, in lateral aspect. (N. C. del.)

smaller in adults from third nymphs than in adults from succeeding nymphal stages and also the humps are less marked in the more feebly developed individuals.

The tarsus of the first leg of the immature stages shows protrusions occupying the positions of the humps on the first tarsus of the adult. This is the case even in the first two stages, as shown in the sketches included in Fig. 3.

(c) The Spiracle. Fig. 4.

In Fig. 4 the outline of the cribriform plate is shown for the following stages, namely first to seventh nymph (*a*) to (*g*); males from third and fifth nymphs, (*h*) and (*i*); and females from fourth and sixth nymphs, (*j*) and (*k*).

The round plate of the first nymph becomes comma-shaped in the second nymphal stage, owing to the formation of an anterior horn. In the next stage, the horn is prolonged ventrally and the plate extends posteriorly. In the fourth and fifth stages the plate develops on the same lines, in the latter case being almost semicircular. In the last two stages, the two ends of the plate tend to close in on one another, the anterior end being directed posteriorly and the posterior end ventrally.

The plate of the adult resembles that of the equivalent nymphal stage, as a comparison of (*h*) with (*d*) and (*j*) with (*e*) in Fig. 4 indicates. The spiracles of adults from fourth (♂) and fifth (♀) nymphal stages are intermediate in structure between those illustrated. Variations in the shape of the plate for any one stage are not very pronounced.

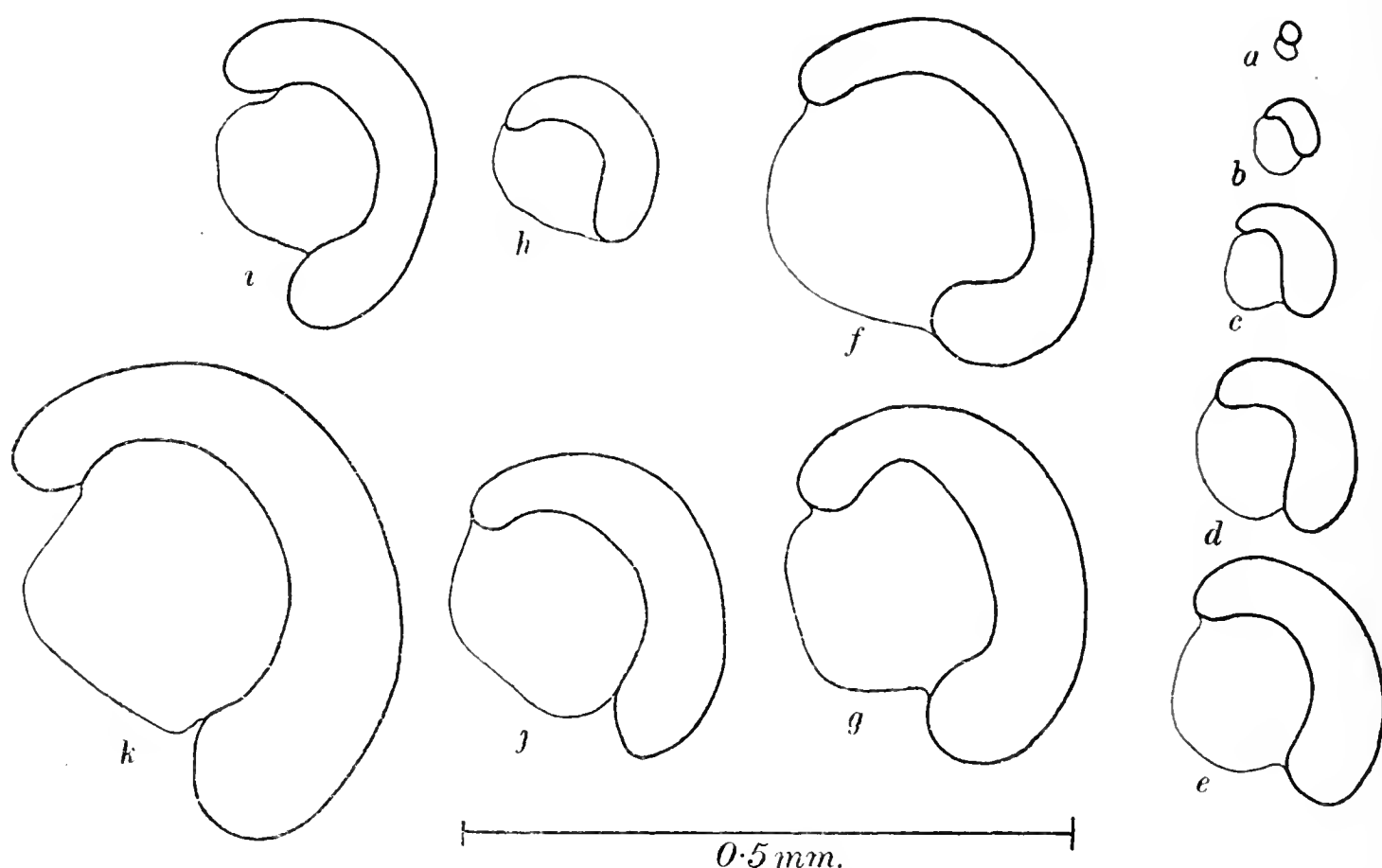
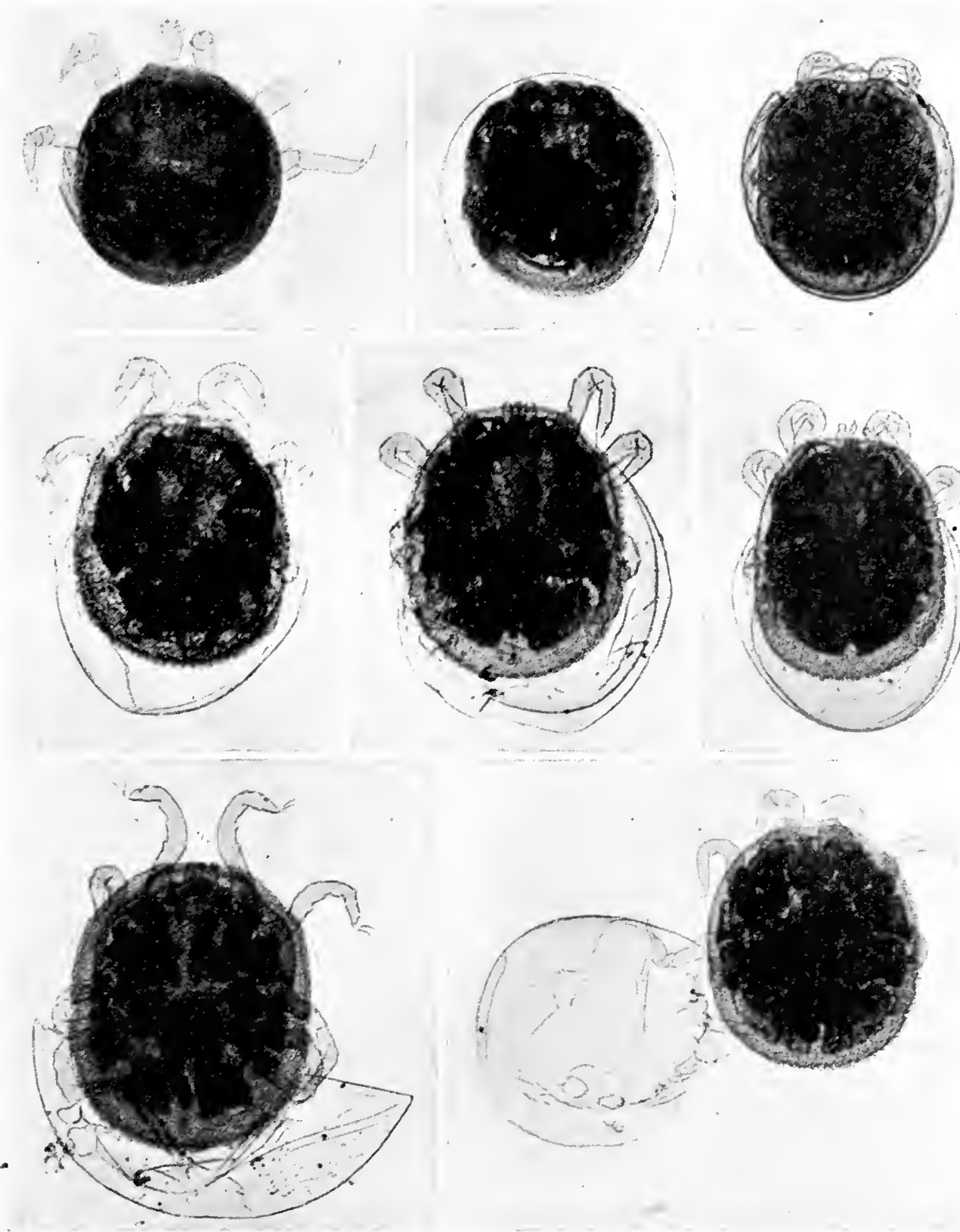


Fig. 4. *Ornithodoros moubata*, outlines of cribriform plates of spiracles: (*a*) to (*g*) of 1st to 7th stage nymphs; (*h*) and (*i*) of males from 3rd and 5th stage nymphs; (*j*) and (*k*) of females from 4th and 6th stage nymphs. Top = anterior, right = dorsum. (N. C. del.)

It is possible therefore, from a consideration of the structure of hypostome, spiracle and fourth tarsus, to determine approximately the stage of development which a tick of this species has attained. The effect of malnutrition in inducing variation is very pronounced in other genera of ticks, as shown by Nuttall (1913) and Cunliffe (1913) and has not been thoroughly studied in this species. The hypostomes show some variation between individuals of the same stage, with regard to the total number and arrangement of the teeth and further study will probably show that the dentition of a small and poorly developed individual will tend to approach that of a normal tick of a preceding stage. Very considerable variation occurs in the tarsal humps of small and normal nymphs of the same stage (*vide* Fig. 3), but as far as was ascertained, the spiracular variation was not very pronounced.



Successive stages of the first nymphal stage emerging from the larval skin. Note the difference in texture of larval skin and egg-shell. (Photograph by G. M. 1912.)

NOTE ON THE EXTERNAL CHARACTERS WHICH SERVE TO DIFFERENTIATE THE SEXES.

By G. H. F. NUTTALL, Sc.D., F.R.S.

THE structures surrounding the genital orifice constitute the only essential external difference between the sexes in *O. moubata*. It is true that the average male is smaller than the average female but some males exceed certain females in size. The smallest males measure 4.5×3 mm.¹, the largest 8.6×6.1 mm. The females likewise vary in size, measuring 6×3.8 to 11.4×9.3 mm. according to our observations, although Dutton and Todd (1905) record a gorged female which attained 12×10 mm. We find that

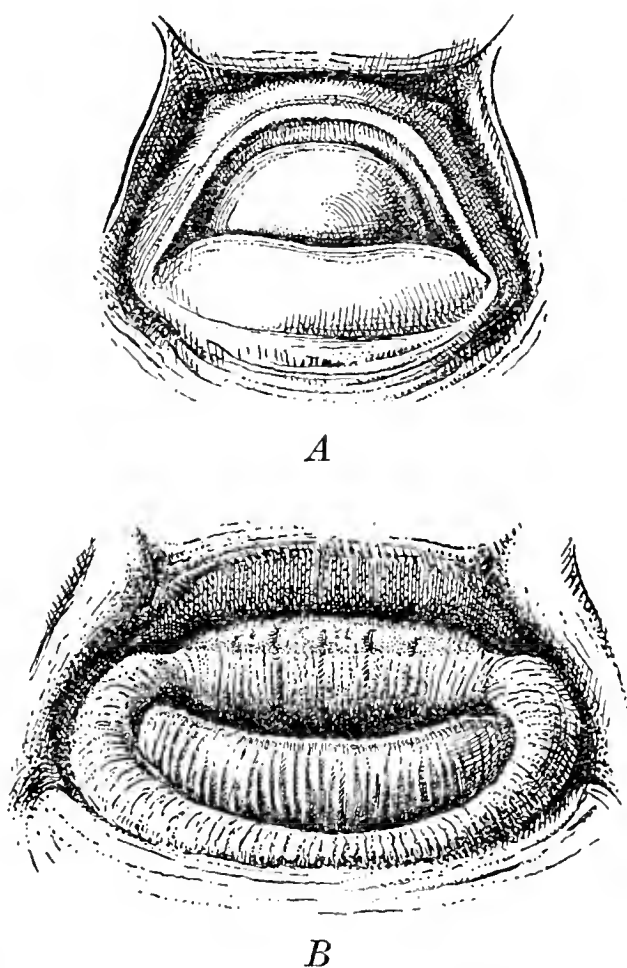


Fig. 5. *Ornithodoros moubata*, genital apertures, (A) of male, and (B) of female individuals.
(E. W. et G. H. F. N. del. 1913.)

the size of the average male is 6.1×4.3 mm. and that of the average female 8×6.5 mm., these measurements being based on the examination of some 400 specimens of both sexes. A variation in size is observable in *moubata* raised under apparently identical experimental conditions. The measurements of the capitulum correspond to those of the body; in small males the capitulum measures 0.95×0.58 ¹, in large females it measures 1.4×0.9 mm.

The genital aperture is usually smaller in the male, but in large males it may exceed the size of the aperture in small females. In the *male* (Fig. 5 A)

¹ The figures give the length \times the width. The length of the capitulum was measured from the posterior ventral margin of the basis capituli to the distal extremities of the extended palps: the width given is that of the basis capituli at the palpal articulation.

the sexual orifice is situated ventrally between the first pair of coxae about midway along their length. The orifice is surrounded by lip-like folds, the anterior lip being concave posteriorly and the posterior lip almost rectilinear. The anterior lip is more highly chitinized than the posterior one. A tongue-like flap arises from beneath the posterior lip and protects the aperture which lies between its anterior portion and the concave margin of the anterior lip.

The *female* genital aperture (Fig. 5 *B*) or vulva, is readily distinguishable, being broad, slit-like, and situated more posteriorly than the male orifice. The vulva lies ventrally in a transverse oval depression of the integument, situated between the posterior angles of the first pair of coxae. The lips of the vulva protrude more or less from the oval depression and are comparable to the lips of the human mouth. The anterior lip is rectilinear on its anterior margin, the posterior lip, with which it is continuous, being concave anteriorly and at the sides where it joins the anterior lip. Protruding in a tongue-like manner from between the lips is a chitinous fold, comparable to the structure seen in the male, and having a concave anterior margin. The female orifice lies between this fold and the anterior lip. In females that are ovipositing the vulva may be situated more anteriorly than here described.

In the last stage nymphs, from which the adults emerge, there is always an Anlage or pit situated at the point where the genital orifice of the adult occurs. This pit may be sufficiently deep to mislead the observer into believing that he is dealing with an adult specimen¹. The Anlage, which is absent in younger stage nymphs, does not however show the structure which is typical of the adult.

SUMMARY OF RESULTS.

1. Copulation between individuals of the two species *O. moubata* and *O. savignyi* may occur, and the stimulus of coition may induce oviposition, the eggs being non-fertile.

2. There is no evidence of parthenogenesis in this species, nor do these ticks undergo ecdysis after reaching maturity.

3. An increase in temperature of 8° C. from 22° C. (*a*) doubles the rate of oviposition, (*b*) decreases the fertility of the eggs by 30 per cent., (*c*) reduces the longevity of the female tick from 715 to 397 days, *i.e.* by 40 per cent. and (*d*) approximately halves the period required for metamorphosis, under laboratory conditions. Under these conditions at 37° C., reproduction is inhibited, and the longevity of the female is reduced by 80 per cent.

4. An individual may undergo from four to eight ecdyses before reaching maturity, the great majority of the males appearing after the fifth ecdysis and the females about equally after the fifth and sixth ecdyses.

5. Engorgement can take place one or two days after emergence, at any stage, the average time required being three-quarters of an hour. There is

¹ The statement by Dönitz (1907) that adult *moubata* may moult is contrary to our experience and is doubtless due to his taking last stage nymphs for adults.

great variation in the extent of engorgement at each stage, but this is not correlated with the ecdysis period. The largest meal may be taken either before or after an individual reaches maturity.

6. Moisture has an adverse influence on the vitality of the individual, excess of moisture inhibiting growth.

7. An approximation to the stage of development attained can be made after the study of the structure of the hypostome, leg and spiracle. The larval and first four nymphal stages are fairly well differentiated, much more so than the later stages, but variation due to nutrition requires further study.

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THE INFLUENCE OF THE HYDROGEN-ION CONCENTRATION IN THE DEVELOPMENT OF MOSQUITO LARVAE.

(PRELIMINARY CONTRIBUTION.)

BY MALCOLM E. MACGREGOR.

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It is a common experience that mosquito larvae when they are brought into a laboratory to undergo their development, often fail to develop normally. At times a high mortality takes place, or the larvae lapse into a condition of suspended development continuing as larvae for months. The latter phase usually ends in death before pupation can take place. I have been puzzled to account for these manifestations for a very long time, and have conducted various experiments in the hope of discovering the cause. The general opinion on the matter, I think, is that there is "something wrong with the food supply," but as a matter of fact larvae supplied with an ample diet often show the same state of affairs.

It appears that what underlies the phenomenon is not the fortuitous absence of certain food elements, but the influence that changes in the hydrogen-ion concentration have upon several factors in the larval development.

In breeding the English tree-hole mosquitoes in this laboratory I found that the larvae, which were kept in the water from the tree-holes without the addition of other water, developed normally. It was at first thought that a high concentration of organic matter in the water was needed by the larvae, and therefore when dilution of the tree-hole water was necessary in order to increase the bulk, a generous supply of beech leaves taken from the tree-holes, and dead insects was added to this water. Tap water was used to dilute the tree-hole water. Nevertheless in every case the development of the larvae failed to continue normally. It was thought that possibly the tap water had some adverse influence, and filtered pond water was used subsequently with no better result.

Soon after an exhaustive series of experiments along these lines had failed to indicate what underlay the phenomenon, it occurred to me to test the various waters in terms of the hydrogen-ion concentration. The local tap water gave a P_H of about 8, and all the waters of the local streams and ponds gave readings of P_H 8.2 to 8.4 approximately. I then decided to test the tree-hole water and I was surprised to find that in every case alkalinity

had given place to acidity, and the readings of the tree-hole water ranged well below P_H 4.4.

This significant result led me to collect a large batch of *Finlaya geniculata* larvae for the following experiment. The tree-hole water in which the larvae were brought to the laboratory gave a reading of P_H 4.4.

The water was poured into jars A and B, an equal number of larvae being placed in each jar.

On the first day of the experiment the P_H of "A" was 4.4, and the P_H of "B" was driven well below 4.4 by the addition of acetic acid.

Ten days later the P_H of jar "A" had risen to 4.6—probably by the absorption of ammonia from the laboratory atmosphere. The P_H of jar "B" was still well below 4.4. By this time the larvae had reached the fourth instar, pupated successfully, and later emerged as very robust mosquitoes. Moreover *none had shown the suspended development condition, and not one had died out of either batch*. This was unusual in my experience, as I had hitherto regarded the dilution of the tree-hole water with tap water as of no consequence provided that dead insects and old leaves were added in abundance. However it has since been found that while fresh dry beech leaves if placed in distilled water will very soon render the water distinctly acid, old beech leaves that have soaked in water for some time lose their acid content.

Old leaves were used in the previous experiments, and it has become evident that they were incapable of neutralising the alkalinity of the added tap water, and by the addition of only a small quantity to tap water the normal acid environment of the larvae was changed to an alkaline environment much to the detriment of the larval development.

These investigations in regard to the development of *Finlaya geniculata* have led me to conduct similar experiments with *Anopheles plumbeus* another tree-hole breeding mosquito. Similar results were obtained with the latter species, *i.e.* the larvae living in acid water flourished, whereas their development in alkaline water was inhibited.

The influence of the P_H on pond and ditch breeding mosquitoes.

I have mentioned that the average P_H of the local ponds and ditches is 8.4—that is, unlike the tree-hole water, the reaction is alkaline.

Experiments were undertaken to determine what effect changes in the hydrogen-ion concentration would have in the case of *Anopheles maculipennis*, *A. bifurcatus* and *Ochlerotatus nemorosus*. It was again found that changing the reaction of the water had a profound effect: acidity affecting development adversely. The following is a typical result of the series of experiments:

About two pints of pond water in which *A. maculipennis* was flourishing under natural conditions was divided equally and placed in three clean glass dishes, marked "A," "B," and "C" respectively. After a small quantity of pond weed had been placed in each dish, and the whole allowed to stand for

some time, P_H readings were taken. A P_H of 8.4 was registered by the water in each dish. By the addition of acetic acid to dish "A" the P_H was altered to 4.4, when tested with methyl red. By the addition of $N/10$ NaOH to the water in dish "C" the P_H was altered to 9.6, when tested by thymol blue.

Sixty larvae of *A. maculipennis* in the pond water in which they had been collected were then stranded on filter paper by pouring the water through the paper. This was done in order that the larvae might be added to the water in the dishes, carrying with them the least possible water so as not to alter the recorded readings. Twenty larvae were then carefully removed from the paper with the aid of a section lifter, and placed in each of the three dishes. The water was again tested by withdrawing a small quantity from each dish to separate tubes, and the readings were found to be the same as before. The three dishes were then placed under equal conditions of light and temperature.

On the following day all the larvae in dish "A" (P_H 4.4) were dead or dying, while the larvae in dishes "B" and "C" were normal and very active. A day later all the larvae in dish "A" were dead, and the sporangia of *Saprolegnia* sp. formed a dense frill around their bodies. The larvae in dishes "B" and "C" still appeared normal, and about ten days later began to pupate, emerging successfully after an interval of a further five to seven days.

Changes in the P_H affecting parasitic Saprolegnia.

Great as is the direct effect of variations in the P_H of the water in which mosquito larvae are living, yet there is another effect that is of the utmost importance to the larvae. This is the influence that variations in the P_H have upon the parasitic fungi of the genus *Saprolegnia* and upon bacterial and protozoal parasites of mosquitoes. I think that it will not be an overstatement to say that of all "natural enemies" *Saprolegnia* is the greatest enemy that mosquito larvae have. *Saprolegnia* is widespread, and anyone who has dealt with mosquito larvae will probably have found that this fungus has taken a heavy toll of the specimens. More than half of the larvae that die in the laboratory while under apparently good conditions are parasitized by this fungus. *Saprolegnia* flourishes in an acid medium, therefore it is rare normally to find *A. maculipennis* and *A. bifurcatus* attacked in the alkaline water of ponds. If, however, the water be brought to the laboratory, its reaction may become reversed owing to the absorption of CO_2 , or if it is intentionally made acid, it will be found usually that most of the larvae will die from being attacked by *Saprolegnia*.

Resistance to *Saprolegnia* attack follows the ordinary laws governing disease in that if the vitality of the individual be high, infection can be successfully combated; if on the other hand the environment in which the individual lives is unsatisfactory to its general health, infection is readily

acquired. For this reason, although *Saprolegnia* flourishes in acid water, it is rare that one finds *Finlaya geniculata*, although living in water that is distinctly acid, parasitized in a natural state, but if the water be rendered less acid by the addition of tap water or even a few drops of $N/10$ NaOH the change in the P_H adversely affects the larvae and they are very often at once attacked by *Saprolegnia*.

While I would not go so far as to say at present that the abnormal development of mosquito larvae in the laboratory is entirely due to changes in the hydrogen-ion exponent, it nevertheless has a profound effect upon the metabolism of the larvae, and their resistance to diseases which affect them. Moreover if their successful development is so adversely affected by changes in the reactions of the water in which they normally live, it may be possible by employing measures that will make the water of ponds acid, and the water of tree-holes alkaline, to find that we have yet another means of combating mosquito development. If so, obviously such means have a limited practical application, governed as they would be by the size of the ponds or other waters to be dealt with.

In the meantime further investigations are proceeding.

My thanks are due to Major H. C. Brown, C.I.E., of the Wellcome Bureau of Scientific Research, for much help that he has given me in the theory and calculations of the hydrogen exponent.

ON THE ZOOLOGICAL STATUS OF THE POLYMORPHIC MAMMALIAN TRYPANOSOMES OF AFRICA AND THEIR RELATION TO MAN.

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(With 1 Text-figure.)

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INTRODUCTION.

THE polymorphic trypanosomes of mammals have commanded a considerable amount of attention during the last 25 years. Bruce's classical work on Nagana in Zululand—conducted in 1895 and 1896—stands out as a splendid example

of far-seeing research into what was then an unopened chapter in Zoology. In this work the trypanosome was studied on the spot, its relations to its insect and mammalian hosts were thoroughly investigated, and the principle of a "natural reservoir" was clearly established. Unfortunately, however, the study of mammalian trypanosomes under natural conditions offers considerable difficulties, and it became necessary to transport the numerous strains, isolated from time to time in various parts of Africa, to European laboratories, where they could be kept up by animal passage and subjected to examination by various methods.

In the course of years many strains of trypanosomes have been collected in this manner, and have been perpetuated by direct inoculation from animal to animal with the syringe in various European laboratories. To these strains various names have been applied by different observers. Numerous tests have been devised to aid in the differentiation of the so-called "species" into which they have been subdivided, and a great deal of controversy has arisen as to the identity of this or that strain. A great stimulus has been given to research on this subject owing to the circumstance that the polymorphic mammalian trypanosomes have a special importance as producers of disease in man himself, as well as in his domestic stock; and also because the susceptibility of laboratory animals to infection with these trypanosomes has enabled them to be studied under convenient laboratory conditions. Consequently, a great mass of literature, dealing with the various "species" of trypanosomes belonging to this group, has now been accumulated.

One of the most striking results of the European work on the polymorphic mammalian trypanosomes of Africa, has been the revelation that, in the course of years, the laboratory strains of these organisms may undergo great changes in morphology, in virulence, and in their immunity reactions. A strain of trypanosomes may thus become, after some years of laboratory upkeep, very different from what it was originally, when freshly isolated from its natural host: and this has led to the splitting up of "species" and consequent confusion in classification.

A further impulse in the direction of splitting up the polymorphic group was supplied by the recognition in recent years of the human trypanosomiasis of Nyasaland. The parasite associated with this disease was said to be distinguishable from all other trypanosomes by the possession of "posterior-nuclear" forms, and as *Trypanosoma rhodesiense* it was duly added to the list of "species." Soon afterwards, however, investigators reported the occurrence of "posterior-nuclear" forms—and incidentally of "anterior-nuclear" forms and other distortions—in other "species" of polymorphic trypanosomes, thus adding to the general confusion.

Until the recognition of the human trypanosome of Nyasaland, the position of man in relation to these protozoa offered no special difficulty. Man was held to be susceptible to, or capable of infection by, one species of trypanosome only—namely, *T. gambiense*: and, accordingly, *T. gambiense* was distinguished

from the other polymorphic mammalian trypanosomes chiefly by its power to survive in man. The speculations which arose concerning this trypanosome were mainly directed, however, towards elucidating the factors determining the spread of trypanosomiasis through native communities—rather than towards the explanation of the origin of the trypanosome itself and its relations to allied “species.” With the appearance on the scene of *T. rhodesiense*, two suggestions regarding its origin were put forward. Bruce and his co-workers in Nyasaland, and Kinghorn and Yorke, held that the newly recognised human trypanosome was merely a race or variety of the widely distributed *T. brucei*—a view to which I have always subscribed. The German investigators, on the other hand, held that *T. brucei* and *T. rhodesiense* were distinct species; and lately Taute has published interesting inoculation experiments on man which, he considers, support the German view.

In nature the great majority of mammalian trypanosomes are transmitted by the agency of insects, of which the most important are the *Glossinae*. The fly can transmit the parasite from mammal to mammal by two methods, namely (a) the direct, and (b) the indirect or cyclical. Direct transmission consists in the mechanical transference of essentially unaltered trypanosomes from host to host; and it may occur when a fly is disturbed in the act of feeding, and completes its meal—within a sufficiently short time—on an adjacent animal. Cyclical transmission, on the other hand, involves complicated developmental changes of the trypanosome inside the fly; and during the earlier stages of this development the insect is not capable of transmitting the parasite to another mammal—i.e., it is not infective.

There is now experimental evidence to show that cyclical development in the fly exerts a steadying influence on the trypanosome, checking tendencies towards variation and keeping it true to type. Moreover, there is every reason to believe that, under undisturbed natural conditions, the cyclical is by far the commoner and more normal method of transmission, in the case of the great majority of the mammalian trypanosomes of Africa.

It thus seems clear that the continued maintenance of a strain of trypanosomes by direct inoculation with the syringe, under unnatural climatic conditions and in hosts which are very different from those of their natural environment, tends to encourage the development, in course of time, of varieties or races differing from the original type. Such artificially propagated strains may, and probably do, lose their power to survive in their normal insect intermediary—the very power, that is to say, which is necessary for their survival and perpetuation in nature. Moreover, it is clear that these artificially produced “strains” cannot be regarded as “species” in the orthodox zoological sense of the term. Zoologists are agreed that “good” species are characterised by their morphological—not merely physiological—peculiarities. If two organisms are structurally identical, at all stages in development, they belong to the same species. But if they display—in addition to such structural identity—differences which are confined to certain physiological features, then

the two organisms are, from the systematic standpoint, "races," "strains," or "varieties" of a single species. Consequently it appears evident that the polymorphic mammalian trypanosomes of Africa should properly be regarded as constituting a single species, which is divisible into a number of more or less distinct strains or varieties. These strains cannot properly be termed "species," for they do not display those constant morphological differences from one another on which the zoological conception of a "species" is based.

Now the artificial propagation of trypanosomes has demonstrated that, under laboratory conditions, the species or strains tend to split up into subsidiary strains; and these may, if the conditions are kept constant, acquire a certain fixity in the course of time. Nevertheless their physiological peculiarities, acquired in this manner, obviously cannot be regarded as characters which are distinctive of the species from which they were originally derived. We are not justified, for example, in using the immunity reactions of laboratory-bred trypanosomes as criteria for their *specific* determination. We cannot maintain that the immunological characters of a strain of "*T. brucei*" which has been kept for ten years in laboratory animals, under artificial conditions, are the standard to which all strains of the species *T. brucei* must conform. We know, indeed, that many of our freshly isolated strains of *T. brucei*—using the name in its historic and valid zoological senses—would not pass such a test of "specificity."

But if the splitting of a species of trypanosome into strains or races can and does occur in the laboratory, then we have some justification for supposing that similar splitting may have occurred, and may still occur, in nature—especially if unusual conditions, comparable with those of the laboratory, are brought into operation. We should expect, from our laboratory experience, that if a trypanosome, which normally inhabits one host, were transplanted into a strange host for many successive generations, then it would undergo modification in some of its physiological characters. Or again, if, in some way, direct transmission by the fly were substituted for cyclical transmission, we should expect to find the trypanosomes undergoing changes similar to those which attend their artificial propagation by the syringe. There is already some evidence that such a natural evolution of races has occurred and is still occurring in the case of the polymorphic mammalian trypanosomes of Africa. The evidence, both direct and indirect, appears to point clearly to the conclusion that all these trypanosomes form a single species, divisible into a number of physiological races—some of which have arisen under natural conditions, others under the artificial conditions of the laboratory: and there appears, on the other hand, to be no good reason for supposing that the so-called "species" into which these trypanosomes have been subdivided by various laboratory workers are true species in the zoological sense. This, very briefly, is the thesis in support of which the following lines are written.

I shall now submit further evidence bearing upon this problem. The evidence presented is all indirect, and includes (1) an examination of the

infectivity of wild lake-shore *Glossina palpalis* and antelope; (2) an investigation of the transmissibility of trypanosomes by laboratory-bred *G. palpalis*, by both direct and cyclical methods; and (3) a study of the effects of different diets on the development of trypanosomes in these laboratory-bred flies. The observations and experiments appear to confirm the conclusions that the polymorphic mammalian trypanosomes of Africa are derivatives of a single species spread over the continent, and that they owe their present differences, great and small, largely to the influence of the mammal upon which, in the course of time, natural conditions have made them dependent as their main blood-host. Further discussion of the general problem will be attempted in the concluding section.

In addition to actual experiments, certain extracts from the literature relevant to the points at issue, and such reflections and conclusions as have arisen from the consideration of the available facts, will be submitted from time to time.

It is to be hoped that the interpretations here advanced will be received in the sense in which they are offered—not as dogmatic conclusions, but as tentative explanations conducive to further research and experiment along practical lines. A theory insusceptible of proof does not serve any useful end in dealing with a problem of such practical importance as the true relationship of the trypanosomes to man. The views put forward below may not be new, but, at any rate, they have not yet been presented with sufficient force to call forth decisive experiments.

At the present time, under the control of Mr Fiske, an organisation is being established in Uganda to recover the long-suspended economic values of the fly-zone by a careful scheme of repopulation. This scheme is based on the assumption that, provided the contact between fly and man be not intimate, there is no danger of a recurrence of human trypanosomiasis in epidemic form.

The fly and antelope on the Islands, where this scheme is being introduced, are still carrying trypanosomes apparently specifically identical with those described by workers in Uganda since 1909, and presumed to be *T. gambiense*. A certain number of natives, canoemen and fishermen, licensed and unlicensed, have, during the last 12 years, been extensively exposed to fly bite in the prohibited area. With the possible exception of two cases, no evidence of trypanosomiasis has been detected in any of them, and it is not absolutely certain that the two cases referred to had not visited the Mpologoma region, where an independent endemic form of the disease has existed for many years, separated from the Victoria Nyanza fly areas by a considerable extent of *palpalis* free country. As will be seen below, there is recent evidence that the trypanosome strains of to-day, in some places at least, are showing characters not before recognised in this fly zone. From the standpoint of the laboratory worker the point of practical importance is to determine as far as possible the effect, if any, of a prolonged sojourn in ruminants on these

lake-shore trypanosomes, with the object of settling their relationship to man. Such investigations are necessarily prolonged and indirect. The decisive experiment of Taute is not possible under British rule. It has been deemed advisable to publish the indications that may emerge from experiments as they occur, and the present paper covers the period from late 1919 to April 1921. It must be pointed out that this fly and trypanosome work, which at Mpumu received my undivided attention, has been carried out at Entebbe in conjunction with the routine work of the laboratory, and other investigations; and this circumstance is my excuse for some of its evident shortcomings.

I wish to express my gratitude to Prof. Clifford Dobell, F.R.S. for valuable suggestions as to the arrangement of this paper, and to Capt. R. L. Sheppard, Librarian to the Tropical Diseases Bureau, who most kindly checked and completed the list of References.

PART I. REFLECTIONS ON THE AETIOLOGY OF THE UGANDA EPIDEMIC.

(a) *Lack of definite evidence of the occurrence of human trypanosomiasis in Uganda lake-shore fly areas before the epidemic.*

The distribution and affinities of the polymorphic organism on the island and mainland shores of Lake Victoria before, during, and after the epidemic are of extraordinary interest. A perusal of Koch's reports of his inspection of the Mwanza and Shirati districts in 1906-7, reveals the existence in 1907 of what is described as an endemic focus of trypanosomiasis, among natives who had never left the district, in the isolated village of Mohurru, to the north of Shirati (Koch, 1906). It is not quite clear whether the word endemic in this connection can be taken to mean a pre-existing focus of long standing and quite independent of the well-known extension from east to west round the shores of the lake.

Again, in a report from the Medical Officer of Health at Kisumu, Dr de Boer, dated 13 December, 1920, describing the occurrence of human trypanosomiasis at Nyakatch near Kisumu, it is stated that the disease had been present in Nyakatch for some 15 years and had spread from Kadimu, where it had existed for a very long time. The cases brought to Kisumu were in an advanced stage of the disease.

Such references suggest that human trypanosomiasis existed in pre-epidemic days along the shores of Lake Victoria. Sir Apolo Kagwa, the Prime Minister of Uganda, has very kindly made exhaustive enquiries for me in the Native Parliament and elsewhere regarding the diseases occurring in Sesse and Buganda before the onset of the epidemic. One result of these enquiries is that I must correct the statement made in 1919 (Duke, 1919 b) that the existence of the word "mongota" in the Buganda language is of itself evidence that an identical or similar disease existed previous to the recognition of sleeping sickness in 1901. The statement was that "there is

no evidence that the word was coined to fit a previously unknown complaint." This is misleading, as the verb "okumongota," meaning to "nod with sleep" did exist, and the derivative "mongota" was applied to the disease because of its most striking symptom.

It appears that two diseases, known respectively as Buko and Kasumagidzi occurred in Buganda Kingdom, but not in Sesse, before the epidemic. Sir Apolo, who of course saw the drift of the questioning, volunteered the remark that a man suffering from Buko seen to-day would be called a case of "mongota." The two diseases were uncommon, especially the latter, and might or might not prove fatal. They were characterised by swellings, especially of the face and neck, great appetite, and drowsiness.

I enquired of Dr Cook at Kampala, whose great experience of native diseases is well known, as to whether he had met with these two complaints in his wide practice. He informed me that although he had not actually encountered them, he was familiar with the name Buko, which, it appears, is the name applied to certain obscure symptoms which the natives say develop as the result of marrying within the prohibited degrees. The other disease, according to native information, results from eating a certain small bird whose characteristic nodding movements are imposed on the unfortunate in whose food an enemy has secretly placed the forbidden flesh. Both diseases are rarer to-day than formerly: they were not in any way associated with residence in tsetse areas, but are rather relics of the superstitions of former days.

The old Basesse questioned were unanimous that no such disease as typical "mongota" occurred on the Islands before the epidemic. They described a "fever" which was distinct from spirochaete fever and which lasted for varying periods—sometimes a few days, sometimes a month or more. Under this heading cases of mild trypanosomiasis might have been grouped. This is, however, pure speculation.

A very interesting fact that has come to light during the course of these enquiries is that at the time of the onset of the great outbreak in Busoga, which coincided with a terrible famine in that country, the Basoga sold their children up to 12 or 16 years of age to the Baganda and the Basesse in exchange for food. As a result, many Basoga actually died in Buganda and Sesse of the new disease which eventually worked such havoc in these two countries.

At the same time, too, natives returning from the great safari¹ of the European trader "Binywera" who went to E. Africa overland round the north-eastern corner of the lake, came home to their as yet uninfected villages to die of this strange disease. The readiness with which my informants, after a few moments' thought, could name the first case which occurred in their own village was most striking, and equally so was the frequency with which this case proved to be either a Musoga slave or a porter from the big safari. The number of Uganda natives originally included in this great safari amounted

¹ Overland journey with native carriers.

to about 6000, of whom very many came from Sesse and the lake-shore region. A very large number died *en route*.

(b) *Acute type of the disease in the early days of the epidemic.*

A perusal of the early Uganda reports (Greig, 1905) reveals the rapid course of the disease in many of the cases examined during the early days of the epidemic, although some patients, such as Kamsasabba, lived for several years. It is to be noticed that at the time of admission most of these acute cases had trypanosomes in their cerebrospinal fluid, though the parasites were not so frequently seen in the blood. Post mortem, the body was often not emaciated. The patient's account of his symptoms usually included headache and general limb pains. In cases 69 GT, a male of 16 years, and 69 FV, a male of 12 years, the disease lasted about six weeks and $3\frac{1}{2}$ months respectively. Case ZM 69 male of 35 years, is described on admission as "appears to be in early stage" on 6. vii. 1904, but he died on 22. viii. 1904. His body was not emaciated. He said he had been ill for three months before admission, and this makes the total course about $4\frac{1}{2}$ months.

Enquiries among old Basesse who lived through the epidemic on the Islands all point to the disease having been very acute in the early days, the limits given being 14 days to 6 months from the time it was first noticed in the sick person. Those lived longest who suffered at the same time from other diseases such as chronic gonorrhoea. This last statement is interesting, as it was volunteered spontaneously and not in response to a leading question.

The virulent nature of the disease in some of these early Uganda cases is thus different from what is ordinarily expected with *T. gambiense* in African natives.

(c) *Direct-transmission hypothesis as an explanation of the virulence of the epidemic.*

In a paper published in 1919 (Duke, 1919 b) a hypothesis was advanced to account for the extraordinary wave of virulent trypanosomiasis which constituted the Uganda epidemic. The contention was that, given a certain degree of contact between *G. palpalis* and man, direct as opposed to cyclical transmission might lead to the development of a strain of enhanced virulence which would persist until it automatically died out with the removal, by death or other causes, of the degree of contact necessary for its propagation. Such a strain when cyclically transmitted, might present a very different degree of virulence. Attention was directed to the drop in the annual death-rate per thousand in the fly area, a drop which had commenced before any depopulation measures were started. To-day we have a flourishing and, according to all accounts, an increasing population along the lake-shore of British East Africa, where no organised measures for depopulation or segregation were taken. The East African coast line suffered very severely in the epidemic. The Germans instituted limited deforestation measures combined with segre-

gation of the sick, and in their territory also, both east and west, the disease died down to a practically negligible factor in the death-rate of the Colony. The direct-transmission hypothesis accounts both for the origin and for the dying down of the virulent form of trypanosomiasis in these at one time populous areas.

A possible factor in the reduction of that broad contact between fly and man which this hypothesis invokes as the essential factor in the epidemic, is afforded by the high lake level of 1906. The effect of the high water of 1917 on the density of the fly was most conspicuous, and led to a definite reduction in their numbers on most of the well-known fly-shores visited by me early in 1918. At the time of my visit the water had again begun to fall, and when Carpenter visited these parts some months later he found but little if any diminution in the fly density. That a considerable reduction did occur, however, there is not the slightest doubt.

Now in Koch's reports (Koch, 1906-07) there occur two interesting observations. Speaking of the distribution of the fly round Mwanza in 1906 he says "The harbour proper of Mwanza is free from Tsetse. This is not in agreement with Feldmann, who claims to have found them especially in the harbour. This contradiction is possibly due to the lake being $1\frac{1}{2}$ metres higher than in previous years." Again, referring to certain women at Kisiba who, in contrast with all the other infected people at that place, had never visited the Uganda fly areas, he says that all attempts to find *G. palpalis* in the places visited by these women failed, the search being made more difficult on account of the high water. These two references are supported by the chart of the lake levels since these were first recorded (Duke, 1919 *a*). The levels of 1906 and 1917 were just about the same; and from analogy alone, we can presume that there was a definite diminution in the numbers of the fly at the time of Koch's visit—a diminution varying in degree in different localities according to the steepness of the shore-line.

This factor, however, can hardly have played any essential part in the diminution in virulence of the epidemic in Buvuma; for, as the death returns indicate, this diminution began in 1903, when the lake level was not high, while in Sesse the change was already noticeable in 1905.

(*d*) *Rôle of cyclically-infected flies in the spread of the epidemic.*

The part played by cyclically-infected flies in the spread of the epidemic is not at all clear. Only a very small number of *G. palpalis* seem capable of sustaining full cyclical development of the parasite, even under the most favourable conditions for picking up trypanosomes. The only available information on the infectivity-rate under the conditions prevailing during the epidemic, are the Entebbe figures given below (cf. Part II, sec. (*a*)). This point is of great importance, and, unfortunately, the number of flies used is small.

A cyclically-infected fly has only to plunge its proboscis into its victim to bring about infection, and in this way the intimate contact between the insect and man demanded by the direct-transmission theory would be favourable to the spread of trypanosomes by cyclically-infected flies. On the other hand, full feeding is presumably favourable to the establishment of trypanosomes in the fly, and in this respect conditions favouring direct transmission are against a high rate of cyclical infectivity.

We have no data on the degree to which *G. palpalis* depended on man for blood in the old days in Uganda, and for even a 0·3 per cent. infectivity rate to be alone responsible for the terrible death roll of the epidemic, this dependence must have been very intimate.

Another factor is the effect of cyclical development on the parasite. It is generally held that the processes involved in this phenomenon serve as a kind of filter through which secondary variations of the trypanosome cannot pass. In other words, we should expect that individual variations in virulence would be steadied down by passage through the fly, at any rate until the characteristic had become firmly established in the course of generations of selection. The extreme virulence of the epidemic in the early days thus seems to require some additional explanation, over and above the part assignable to cyclically-infected flies.

The most commonly advanced explanation of the origin of the Uganda epidemic seems to be the introduction of trypanosomes from outside, rather than the sudden acquisition of increased virulence by a previously existing organism.

Whatever the origin, the two factors which challenge our attention are the remarkable outburst and spread of a virulent disease and its equally remarkable dying down, which was first manifested by the death-returns, years before any steps were taken to remove the populations. Nature adjusted matters for British East Africa where the populations have been increasing for years, but where cases of chronic trypanosomiasis are still to be found.

The direct-transmission hypothesis supposes the development of a strain of enhanced virulence as the result of continued transmission unaccompanied by the stabilizing influence of cyclical development in *Glossina*. As a means of spread the direct method is more rapid than the normal one, as the trypanosome makes less demand on the tsetse. Removal of the intimate contact between fly and man necessary for the free operation of direct transmission, would lead to the disappearance of this hypothetical virulent strain.

As will be seen below it is probable that direct transmission depends for its success on the presence of trypanosomes in fair numbers in the blood, and this phenomenon is usually a manifestation of a virulent disease which will thus be especially suited to this method of propagation. Chronic disease, on the other hand, with few parasites in the blood, will not lend itself to direct transmission; but, if we may judge by analogy with antelope infections, such cases may be well suited to cyclical development in the fly.

Mosquitoes, etc. Whether or not mosquitoes played a part in the spread of human trypanosomes in Uganda we do not know. The French observers attach considerable importance to their intervention in the transmission of sleeping sickness in the Congo.

On many of the islands culicine mosquitoes are very common. Bagshawe has shown, however, that the probabilities are against this factor having been of importance in Uganda; and the absence of any instance of direct transmission by insects other than *Glossinae* in the community of infected and healthy monkeys at the Entebbe laboratory—which presents a very fair experimental parallel to the conditions in an infected village, as far as mosquito and *Stomoxys* carriers are concerned—is of interest in this respect.

PART II. INFECTIVITY OF WILD LAKE-SHORE FLIES, 1920–21.

The early Sleeping Sickness Commissions of the Royal Society in Uganda established the fact that freshly caught wild lake-shore *G. palpalis*, fed upon clean monkeys, infected the monkeys with a trypanosome indistinguishable from the organism responsible for the human disease. Since that time various observers have shown that the flies have maintained their infectivity in spite of the wholesale removal of human beings from their reach.

The following section deals with the infectivity of the wild *G. palpalis* on Victoria Nyanza, past and present, as manifested by feeding experiments and, more accurately, by actual dissection.

A brief reference is made to the infectivity of wild *G. morsitans* in Uganda and Nyasaland.

In these experiments attention has been devoted more especially to the polymorphic trypanosome which utilises the gut and salivary glands of the fly. In the feeding experiments the incubation period in the monkey is reckoned as seven days, and any flies which may have been fed on the monkey during this period are ignored in making the count. It is assumed that only one infective fly occurs in each positive experiment.

A *résumé* of previous experiments on the lake-shore infectivity in Uganda is given for comparison. It must be remembered that in these earlier experiments there was no question as to the identity of the trypanosomes obtained with *T. gambiense*.

(a) *Feeding experiments with wild flies.*

(a) Entebbe shore. May–July, 1903. Natives present, infected 1 in 3 or 5. No antelope.
(First R.S. Commission.)

Total flies fed	989–1360*
Number of infections	3
Percentage of infective flies	0.30–0.22.

* In these experiments the monkeys were only examined at intervals of seven days. As batches of flies were being fed daily it is, therefore, impossible to estimate accurately the number responsible for each infection.

(b) Entebbe shore. Sept.–Nov. 1903. No natives. No antelope. (First R.S. Commission.)				
Total flies fed	1421
Number of infections	1
Percentage of infective flies	0.07.
(c) Entebbe shore. June–Sept. 1904. No natives. No antelope. (First R.S. Commission.)				
Total flies fed	2299
Number of infections	0
Percentage of infective flies	0.00.
(d) Mainland. Nov. 1909–July 1910. (Bruce's Commission.)				
Total flies fed	28274
Number of infections	18
Percentage of infective flies	0.06.
(e) Mainland. Aug. 1910–Feb. 1911. (Fraser and Duke.)				
Total flies fed	51078
Number of infections	5
Percentage of infective flies	0.09.
(f) Mainland. Jan. 1920–Jan. 1921.				
Total flies fed	6441
Number of infections	2
Percentage of infective flies	0.03.
(g) Damba Island. Jan.–June, 1910. (Bruce's Commission.)				
Total flies fed	6356
Number of infections	2
Percentage of infective flies	0.03.
(h) Damba Island. May, 1911. (Carpenter.)				
Total flies fed	885
Number of infections	1
Percentage of infective flies	0.11.
(i) Damba Island. Sept.–Dec. 1920.				
Total flies fed	3732
Number of infections	3
Percentage of infected flies	0.08
(j) Sesse Islands: Bugalla and Kome. Late 1911 and early 1912. (Carpenter.)				
Total flies fed	12000
Number of infections	2
Percentage of infective flies	0.06.
(k) Sesse Islands: Nsadzi and Kimmi, before arrival of antelope. 1910–1911. (Fraser and Duke.)				
Total flies fed	14209
Number of infections	0
Percentage of infective flies	0.00.
(l) Sesse Islands: Nsadzi and Kimmi, after arrival of antelope. May–July, 1914. (Carpenter.)				
Total number of flies fed	2076
Number of infections	1
Percentage of infective flies	0.04.

The highest infectivity rate in these experiments is that obtained when a heavily infected native population was living in the fly zone at Entebbe in 1903, but in these experiments, as only flies which obviously contained blood were counted as having fed, it is probable that the percentages are too high. Since that time the infectivity level has remained remarkably constant. On

Damba the percentage is just a little higher than formerly, but the totals are too small to warrant any conclusion.

The effect of the settlement of antelope on an island previously free from these animals and carrying only hippopotamus, reptiles, otters, and birds is well shown by the two Nsadzi experiments (*k*) and (*l*).

The small number of flies infected with the polymorphic organism is striking, and should be compared with the figures obtained with laboratory-bred flies set forth in Section V.

(*b*) *Dissections of wild flies.*

Mainland. Nov.-Dec. 1920. Proboscis, glands, and gut examined.

Total dissected	299	(♂ 184, ♀ 115)
Flagellates in gut only	28	<i>i.e.</i> 9.3 % (♂ 20, ♀ 8)
„ proboscis or glands	0	

Zinga Island, near the mainland. Dec. 1920.

Total dissected	48	(♂ 10, ♀ 38)
Flagellates in gut only	10	<i>i.e.</i> 20.8 %
„ proboscis or glands	0	

Sesse Islands other than Damba. Dec. 1920.

Total dissected	307	(♂ 263, ♀ 44)
Flagellates in gut only	15	<i>i.e.</i> 4.8 % (♂ 14, ♀ 1)
„ proboscis	3	<i>i.e.</i> 0.9 %
„ gut and glands	0	

Damba Island. Sept. 1920. Gut and glands only dissected.

Total dissected	490	(♂ 423, ♀ 67)
Flagellates in gut only	0	

Of these flies all save 183 had fed over-night on a clean monkey.

Damba Island. Dec. 1920. Gut and proboscis dissected, glands examined only if any flagellates seen.

Total dissected	695	
Total containing flagellates	30	<i>i.e.</i> 4.3 %
In gut only	2	<i>i.e.</i> 0.28 %
In proboscis only	27	<i>i.e.</i> 3.8 %
In gut and glands	1	<i>i.e.</i> 0.14 %*

* The monkey fed upon by this fly became infected.

Of the above flies all save 117 had fed over-night on a monkey.

Total percentages for Damba Island: 1185 flies.

Gut only	0.16 %
Gut and glands	0.08 %

In the dissections, as with the fly-feeding experiments, the main object was to determine the proportion of flies infected with the polymorphic trypanosome. Proboscis infections give an additional indication of the extent to which the flies feed on antelope.

All the infected proboscides showed flagellates in the hypopharynx, and usually a few loose in the labrum. In four flies there were big fixed clusters in the labrum.

Of the gut-only infections a certain number were due to *T. grayi*, but all the positive flies were not examined in stained preparations.

Infectivity data obtained by dissection are, of course, more exact than those from fly-feeding experiments. It is interesting, however, to note that the same figure for the infectivity of wild Damba flies in 1920 is obtained by fly-feedings as by dissection, *i.e.* 0.08 per cent.

The observations set forth in subsections (a) and (b) show that the infectivity of the wild *G. palpalis* of the Uganda lake-shore to the polymorphic trypanosome has remained remarkably constant throughout the last 12 years.

(c) *G. morsitans* dissections in Uganda and Nyasaland.

The following figures were obtained by the dissection of *G. morsitans* in the Northern Province and are given for comparison (Duke, 1916):

Total dissected	1117
Flagellates in proboscis only	8.5 %
„ proboscis and gut	3.1 %
„ gut only	1.6 %
„ gut and glands	0.17 %

In Nyasaland the Commission working with *G. morsitans* found the flies infected as follows (Bruce, 1914 b):

1912—Total dissected	1975
Flagellates in gut and glands	0.1 %
1913—Total dissected	1060
Flagellates in gut and glands	0.09 %

The infectivity with the polymorphic trypanosome, as revealed by fly feedings in the Northern Province and Nyasaland respectively, was 0.59 per cent. and 0.2 per cent.

(d) *The trypanosomes of palpalis* regions in Uganda and elsewhere.

The absence of the *nanum-pecorum* group of trypanosomes from the wild-fly of the Uganda lake-shore is remarkable. In the course of dissections made in the inland districts of the Protectorate, *T. nanum* was detected in 1.4 per cent. of 713 *palpalis* dissected from the Northern Province, but was not found in 477 flies from the Western Province (Duke, 1913 d). At Mpumu *T. nanum* was transmitted by laboratory-bred flies, but both this trypanosome and its near ally *T. pecorum* seemed to find great difficulty in developing in this tsetse (Duke, 1912 a, and Fraser, 1912). It is almost certain that these trypanosomes were introduced into Sesse in cattle in the old days, together with *T. brucei*. After the raids of 1895 into Bukedi, cattle from this *morsitans* area reached the islands; the trypanosomes have, however, not established themselves. Proboscis-only infections amounted to 3.0 per cent. in the Northern Province fly but were not seen in flies from the Western Province.

No trypanosomes of the polymorphic group were obtained from 1333 *palpalis* tested by feeding in the Northern Province. In the Western Province,

507 *palpalis* caught on the now uninhabited shores of Lake Kiraro near Lake George, infected a clean monkey with a trypanosome of the *brucei* group. There is a possibility that a few *pallidipes* may have been included in these flies from Kiraro, where game of many kinds is very common. I hope to revisit this locality and settle the important point whether *palpalis* is here carrying a *brucei*-like organism.

Palpalis regions elsewhere in Africa are characterised by similar trypanosomes, and often, in addition, by the human parasite *T. gambiense*. The strains isolated by Dutton and Todd in the Congo from game and stock doubtless included representatives of all three groups, although these observers held that only one species was present. In those days the term *T. dimorphon* seems to have included several different species, including doubtless *T. uniforme* and the *nanum-pecorum* group.

The reactions of the polymorphic trypanosomes recovered by Dutton and Todd from game and stock were of the chronic *gambiense* type; they were held by these observers to be identical with *T. dimorphon* (Dutton, 1907).

In Principe, where *palpalis* occurred under unique conditions, the same organisms were recovered from fly and cattle, and here again the representative of the polymorphic group gave the laboratory reactions of *T. gambiense* (Da Costa, 1916).

In Uganda, therefore, the trypanosomes most commonly carried by *G. palpalis* are *T. vivax*, *T. uniforme* and the polymorphic organism referred to throughout this paper. Broadly speaking, where *T. brucei* or *T. pecaui* are found in *palpalis* areas in Africa another tsetse species will be found to occur in that area. As will be seen later there appears to be an important exception, the significance of which will be discussed later on. Trypanosomes of the *nanum-pecorum-congolense* type occur in the *palpalis* of certain inland areas, but not of Lake Victoria.

PART III. ANIMAL REACTIONS OF THE UGANDA LAKE-SHORE POLYMORPHIC TRYPANOSOMES.

An important difference between freshly isolated strains of *T. gambiense* and of *T. brucei* has always been the relative chronicity of the former in laboratory animals.

In this section the animal reactions of the present-day lake-shore trypanosomes of Uganda are considered, the organisms being grouped according to their provenance into wild-fly, human, and antelope strains.

In the subjoined tables M, S, D, P and R stand for monkey, sheep, dog, guinea-pig and rabbit respectively. No rats were available.

(a) *Wild-fly strain, Mainland.*

This strain was recovered from wild lake-shore flies from the mainland near Entebbe. The reactions in monkeys are shown in Table I.

Table I.

Animal and ex- periment number	Strain of infecting trypanosome: number in direct transmission series			Duration of disease in days	Remarks
M 43	Wild flies			63	Medium sized animal
M 59	Cycle by lab.-bred flies from M 43			409	„ „
M 63	1st passage, direct transmission			79	“Nakabugo” species
M 72	1st	„	„	77	Large animal
M 88	2nd	„	„	Alive after 449	Thin but active: still shows T.’s; large animal
M 93	3rd	„	„	47	Poor conditioned animal
M 98	3rd	„	„	Alive after 430	Thin but active: still shows T.’s; medium size
M 107	4th	„	„	140	Medium size
M 111	5th	„	„	90	„
M 117	6th	„	„	125	Large size
M 124	2nd	„	„	91	Medium size
M 142	7th	„	„	Alive after 333	„
M 132	8th	„	„	254	„
M 140	9th	„	„	94	Well-nourished, seemed well day before death; medium size
M 147	10th	„	„	92	Medium size
M 161	11th passage by blood inoculation			Alive after 225	Medium size: since dead
M 139	3rd passage: cycle, lab.-breds off 88			31	Small, ill-nourished; T.’s + + + throughout disease
M 160	4th passage, direct transmission from 139			Alive after 346	Small size
M 164	5th passage, direct transmission from 160			Alive after 298	Medium size
M 169	12th passage: cycle, lab.-breds off 161			74	“Nakabugo” species
S 286	Inoculated from M 88			Alive after 111	
S 287	„	„	„	Alive after 111	
D 281	„	„	M 59	31	Corneal opacity after 14 days. Few posterior-nuclear forms seen on penultimate day of disease
D 282	„	„	„	40	Corneal opacity after 14 days
P 277	„	„	M 88	Alive after 122	Posterior-nuclear forms present
P 278	„	„	„	Alive after 122	„ „ „
P 244	„	„	M 59	Alive after 117	Posterior-nuclear forms rare: since dead
P 245	„	„	„	Alive after 117	Posterior-nuclear forms rare: since dead

The average duration of the disease in 12 completed monkey experiments with the common green species was 216 days. None of the monkeys showed any marked symptoms, and somnolence was only noticeable in the very late stages.

The white-nosed *Cercopithecus*, known locally as “nakabugo,” is much less robust in captivity than the green monkey; in infected monkeys of this

species somnolence was a much more pronounced symptom, and commenced earlier in the disease. Unless otherwise stated all the monkeys quoted in these tables are the black-faced green-coated *Cercopithecus* sp. The sheep were the local native fat-tailed variety.

The average duration in two dogs was 35 days.

Table II. *Wild-fly strain, Damba Island.*

Animal and experiment number	Strain of trypanosome	Duration of disease in days	Remarks
M 207	Damba flies direct	30	No facial oedemas
M 208	„ „	133	„ „ emaciation marked
M 209	„ „	92	„ „
M 272	Inoculated from 207	82	Emaciated. For 10 days of the disease showed cloudiness of both corneae which advanced to total blindness and then slowly cleared again. This symptom never before noted in Uganda
P 240	Inoculated from 207	56	Posterior-nuclear forms seen
P 241	„ „	Alive after 158	„ „

Average duration of disease in four completed monkey experiments was 84 days.

(b) *Examination of situtunga (Tragelaphus spekei) on the Sesse Islands.*

In November 1919 defibrinated blood of 12 situtunga was inoculated into clean monkeys without any trypanosomes being recovered. The animals were shot mostly on Bugalla Island, the largest of the Sesse group, where the clearing of the fly-shore brought about by the feeding of innumerable antelope is most marked. Slides from one of these animals showed *T. vivax*. None of these antelope was shot on Damba.

In September 1920 two monkeys were inoculated, each with the citrated or defibrinated blood of six situtunga shot on Damba Island. Of these five were young animals. One of the monkeys remained negative to daily examination for six weeks. The other, nine days after the first inoculation, showed in its blood the polymorphic trypanosome which is referred to throughout this paper as the 1920 antelope strain.

The reactions of this antelope strain will now be discussed.

(c) *Damba antelope strain*, 1920.

Table III.

Animal and ex- periment number	Origin of trypanosomes	Duration of disease in days	Remarks
M 173	Original antelope injection	66	Medium size: puffy eyelids
M 171	Inoculated from M 173	38	„ „ „
M 197	„ M 171	38	Large size: puffy eyelids
M 227	„ P 198	81	„ „ „
M 122	Cycle, lab.-breds off M 173	44	Medium size
M 194	„ „ M 173	56	Small size
M 250	„ „ M 227	31	Medium size: puffy eyelids
M 219	„ „ D 232	42	„ „ „
M 275	„ „ D 232	78	Medium size: female with young at breast. Slight puffy eyelids
M 221	„ „ M 227	56	Medium size
P 198	Inoculated from M 171	47	No obvious external symptoms
P 231	„ „ P 198	36	„ „ „
D 232	„ „ P 198	25	Corneal opacity late in disease
D 273	„ „ M 227	25	Corneal opacity early symptom
D 295	„ „ M 227	18	„ „ „
D 296	„ „ M 219	21	„ „ „
R 176	„ „ M 173	34	Facial oedema: purulent blepharitis
R 177	„ „ M 173	37	„ „ „
R 199	„ „ M 171	28	Facial oedema: purulent blepharitis; received 1.75 c.c. human serum half an hour before trypanosome injection
R 200	„ „ M 171	42	Facial oedema: purulent blepharitis
R 270	„ „ M 227	58	Head oedema: blepharitis
R 270 a	„ „ M 227	22	Slight head oedema: no blepharitis
S 283	„ „ M 227	45	
S 284	„ „ M 227	37	
S 285	Infected by positive fly exp.	61	

In *monkeys* the disease was characterised by puffiness of the upper and lower eyelids which was noticeable quite early in the disease. Emaciation was fairly rapid. No corneal changes were noted, but there was some photophobia. In no case were posterior-nuclear forms seen in the infected monkeys. The average duration for 10 completed monkey experiments was 53 days.

In *guinea-pigs* there were no obvious symptoms; posterior-nuclear forms were common. Average duration 41 days.

In *dogs* the disease was characterised by rapid emaciation and the early onset of cloudiness of the cornea, which led to complete blindness. Prolonged search in slides taken late in the disease revealed very rare posterior-nuclear forms in D 295. Average duration 22 days.

In *rabbits* the usual symptoms of head oedema and blepharitis occurred: no posterior-nuclear forms were seen. Average duration 33 days.

In *sheep* (three experiments) the average duration was 48 days.

The acute nature of the disease in dogs characterises all the strains, being most marked with the antelope strain both as regards the rapidity of the disease and the early onset of the corneal symptoms.

The behaviour of the human strain is, however, very different. In monkeys the incubation period is prolonged and trypanosomes are always rare in the blood. Four attempts to infect guinea-pigs, and two inoculations of rabbits failed to produce infection.

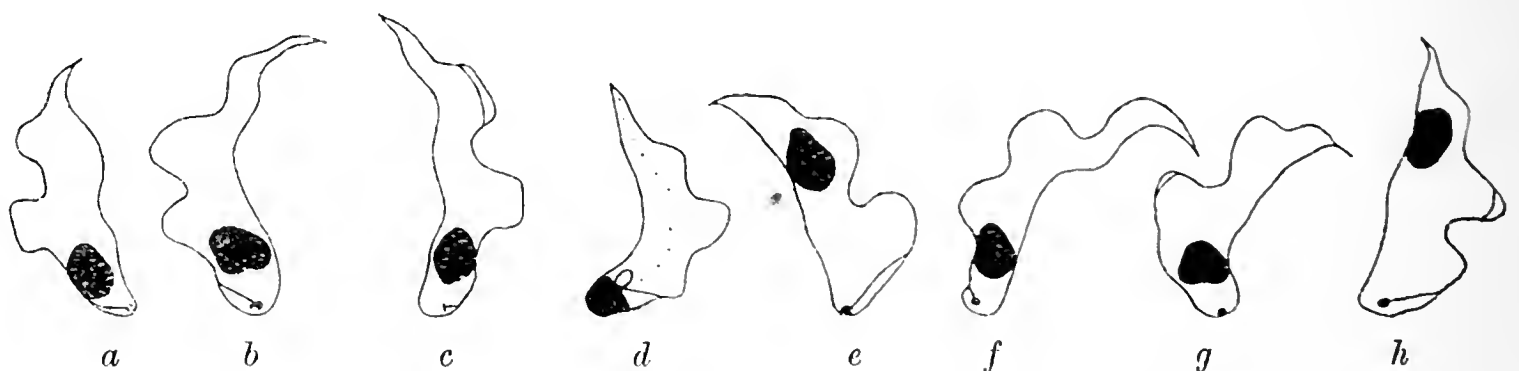
Baboons, incidentally, appear to be immune to both strains; laboratory-bred flies, proved to be infective before and after, carrying the human and wild-fly strains, were fed on baboons without any sign of infection resulting in either case. Both animals were examined by daily blood examination of stained thick and of fresh films, and, in the case of the human strain, by sub-inoculation.

When first isolated in the original monkey (M 173) this 1920 antelope strain appeared to be characterised by peculiar movements, distinct from those of the mainland wild-fly strain. The long forms showed a definite translatory movement, progressing in a steady snake-like manner across the field. Subsequent investigation has, however, shown that while this movement is more often seen with the antelope strain, it is also exhibited by the wild-fly strains in various animals.

(d) *Posterior-nuclear forms.*

The appearance of definite posterior-nuclear forms in guinea-pigs inoculated with these strains is of considerable interest. Such forms have not been hitherto described from the Uganda lake-shore fly area, though Blacklock found them in a European strain of the Uganda *T. brucei*, which, however, did not emanate from the lake-shore fly zone (Blacklock, 1912).

These forms were most numerous in guinea-pigs infected with the Damba antelope strain. Thus in P 198, on the day before its death, posterior-nuclear forms amounted to 15·2 per cent. of the short individuals counted, these short forms predominating in the blood, while trypanosomes with the nucleus anteriorly displaced amounted to 4·2 per cent. In P 240, Damba fly strain, posterior-nuclear forms were rarer, but a day or two before death amounted to 3 per cent. of all trypanosomes present, while a few anterior-nuclear forms were also seen.



×2000. Camera lucida, *e* and *f* from guinea-pig 241, others from guinea-pig 198.

P 277, infected from M 88, *i.e.* direct transmission of mainland wild-fly strain, showed, in 100 individuals counted, seven with the macronucleus in the posterior, and four with it in the anterior quarter of the body.

Thus the antelope strain, the mainland fly strain, and the Damba wild-fly strain have shown posterior-nuclear forms in guinea-pigs; and the mainland fly strain, after one cyclical passage through laboratory-bred flies and sub-inoculation into D 281, showed a single example after prolonged search in a slide swarming with trypanosomes.

(e) *Action of human serum.*

The 1912 Damba antelope strain showed no response to human serum.

With the 1920 strain a few controlled inoculation experiments into guinea-pigs and rabbits were performed in which the serum was either mixed with the inoculum and injected at once, or else injected half an hour before the trypanosomes. No protective action was observed.

Experiments *in vitro*, with and without the addition of fresh guinea-pig complement and at room and incubator temperature, also revealed no action on the trypanosomes by either human, mangabey, or lizard serum.

Similar experiments with the Damba fly strain were also negative.

(f) *The behaviour of a black mangabey (Cercocebus albigena, sub sp.), obtained from the Kyagwe forests, towards the trypanosome used in these experiments.*

This species, of which there were three individuals at the laboratory, has proved very resistant to the various strains used. Two out of three have shown trypanosomes in their blood at rare intervals during prolonged daily examination both by fresh and stained thick film. The history of the three individuals is as follows:

- No. 114. (a) Interrupted feedings daily by 35 flies off Monkey 59 from 24. iii.–8. iv. 20.
 (b) Fed upon by positive flies of boxes 24 and 25 on 27. v.–29. v. 20.
 (c) Trypanosomes seen in very small numbers in monkey's blood on 19. vi. 20.
 (d) Fed upon by clean laboratory-bred flies from 20. vi.–28. vi. 20. 85 dissected between 14th and 26th days after first feed: none found infected.
 (e) Negative to daily examination from 20. vi.–17. x. and from 20. x. 20.–5. iii. 21. Still alive and well.
- No. 112. (a) Interrupted feedings off Monkey 72 with 35 flies from 22. iii.–30. iii. 20.
 (b) Fed upon by positive box 77 from 9. xi.–10. xi. 20.
 (c) Trypanosomes first seen in blood in very small numbers on 21. xii. 20. Between 22. xii. 20 and 26. ii. 21 trypanosomes seen on 13 of the daily examinations.
 (d) Fed on by clean laboratory-bred flies from 24. xii.–29. xii. 20. 130 flies dissected after 7th day all nil: also from 29. i.–17. iii. 21 by other laboratory-bred flies of which 36 were dissected after 7th day; none of these flies was infected. Total 166. Still alive and well.
- No. 86. (a) Fed upon by 1171 wild lake-shore flies before its peculiar resistance was realised.
 (b) Fed upon by positive boxes 51, 52, 53 on 9. viii.–11. viii. 20.
 (c) On 24. i. 21 inoculated with 1.5 c.c. citrated blood of Monkey 227 which showed trypanosomes (buck strain).
 (d) Negative to trypanosomes in stained thick and fresh films from 30. iii. 20 to 13. ii. 21.
 (e) Died 16. ii. 21. Spleen hard and small: blood inoculated half an hour after death into clean monkey which did not become infected.

(g) Infection of chimpanzee.

An adult male chimpanzee which had lived for some three months at the laboratory was fed upon by positive-fly boxes 48 and 49 on 4-7. viii. 1920. These boxes were proved to be infective to clean monkeys both before and after these dates, and on subsequent dissection a fly with trypanosomes swarming in gut and glands was found.

Trypanosomes were first seen in the blood of the chimpanzee on 12. viii. 1920. On 8. vi. 1920 and on 10. vi. 1920 malaria parasites were found in the animal's blood; the parasites were only seen in stained thick films and were present as fairly large rings. No crescents were seen. The animal had definite rigors and pyrexia and was plainly inconvenienced by the plasmodial infection.

Early in November he began to go off his feed a little, and on the 15th oedema of the left eye appeared, lasting, however, only 24 hours.

The animal was affectionate and intelligent, but was obviously suffering. There were no signs, apart from the oedema, of trypanosome infection, and the appearance towards the end of November of commencing incontinence of urine together with the general look of the animal suggested some abdominal lesion.

Unfortunately this chimpanzee absolutely refused to allow any kind of examination to be carried out without a struggle which invariably resulted in some one getting more or less severely hurt. All attempts at pricking the finger or scratching the ear were met with determined protest and resistance, and, if persisted with, made him thoroughly upset and miserable. I was therefore compelled to let him alone save for occasional examinations, and towards the end of his illness he was not handled at all.

He died on 30. i. 1921 without any signs of cerebral involvement, somnolence, or further oedemas.

Post-mortem the body was emaciated and the incontinence of urine had led to some excoriation around the pubis. The liver was large, pale and fatty; the spleen not enlarged, firm, and free from infarcts or abscesses. Kidneys healthy. Much fat around heart, which was otherwise normal. Abdominal viscera: small intestine normal; large intestine, terminal portion above rectum much ulcerated, many adhesions in lower abdomen, matting gut coils together and to bladder; 10 inches above the anus a perforation $\frac{1}{4}$ inch in diameter through which a small nematode was protruding into the peritoneal cavity. On opening up the bowel, the mucous membrane was found to be much congested and showing many small ulcers, some healed and with dark pigmented edges. Many of the adhesions of long standing. Large quantity of bright yellow fat in the omentum. No large glands were noticed save in the mesentery. Bladder contracted.

The cause of death was plainly chronic peritonitis with perforation and extensive ulceration of the lower portion of the large intestine.

Whether or not the animal was seriously inconvenienced by the trypanosome it is impossible to say: certainly he gave the impression of feeling the malarial attack more than the subsequent trypanosome infection.

The strain employed in this experiment was the wild-fly organism from Monkey 88, which represented the second passage by direct transmission.

There are at present two other chimpanzees at the laboratory, the younger of which a month or so after its arrival developed an attack of malaria in which the parasites appeared to be identical with those seen in the original chimpanzee. The attack thoroughly upset the little animal for three or four days, after which he renewed his interest in life. The other animal is an old male, grey-haired all over his body and with what appears to be commencing arcus senilis. He allows no liberties to be taken with him at present, and no examinations have as yet been made of his blood.

(h) Conclusions.

The animal-reaction experiments of this section show:

(a) That the situtunga antelope on Damba Island at the end of 1920 harboured a polymorphic trypanosome whose characteristics are different in several respects from those of the polymorphic organism isolated from the same antelope species on Damba in 1912 (Duke, 1912 *b*). Whereas the 1912 strain agreed with *T. gambiense*, the 1920 strain shows many of the characters usually assigned to *T. brucei*.

(b) That the disease in monkeys and in sheep caused by the Damba antelope strain of 1920 is on the whole more virulent than that produced in these animals by the wild-fly strain from the mainland; the trypanosome strain derived from wild Damba *G. palpalis* occupies an intermediate position as regards its pathogenicity towards monkeys.

(c) That the general virulence towards laboratory animals of the Damba antelope strain of 1920 is, on the average, greater than that shown by the other two strains investigated.

(d) That posterior-nuclear forms have been developed by all the three strains, but appear to be more frequent with the Damba antelope strain.

(e) That the virulence of the human strain, recently isolated from a native who was probably infected in the Mpologoma endemic area, is very much less towards monkeys, guinea-pigs and rabbits. It was found difficult to bring about the infection of monkeys with human blood containing living trypanosomes, and subsequent passage in these animals was always characterised by a long incubation period and, during the first six months at any rate, rarity of the parasites in the peripheral blood. Several attempts to infect guinea-pigs and rats from first and second-passage monkeys failed. No posterior-nuclear forms were ever seen in blood slides from the animals infected with the human strain.

(f) That the baboon is immune to the human, the antelope, and the wild-fly strains of trypanosomes, whether infection be attempted by the

syringe or by positive flies. A black mangabey species is extremely tolerant to these organisms. The chimpanzee is susceptible to infection by the wild-fly mainland strain; unfortunately, owing to an independent abdominal infection which proved fatal, it is not possible to report on the course of the disease in this animal.

PART IV. DIRECT-TRANSMISSION EXPERIMENTS.

These experiments were undertaken to test the hypothesis advanced by me in 1919 that this mode of transmission may play an important part in the production of strains of enhanced virulence, when the natural conditions result in the predominance of direct over indirect or cyclical transmission.

It soon became evident, however, that a considerable time must elapse before any opinion can be advanced as to modification in the virulence of the directly-transmitted strains.

In these experiments, boxes containing 30-35 flies were applied for a quarter of a minute or so to the infected monkey and then transferred to the clean monkey for a slightly longer period; the process was then repeated immediately, so that each day the flies had two "interrupted feeds." The flies were finally fed on the clean monkey as required, to keep them alive.

At first wire-sided boxes were used, later on mosquito-net sides were substituted for the wire to ensure more rapid feeding by the tsetse.

Table IV sets forth the direct-transmission experiments.

The reason for the repeated applications of the boxes was that the main object of these experiments was infection of as long a series of monkeys as possible, to see whether there ensued any alteration in virulence in the later passages.

Allowing for a prolonged incubation period, which it is clear from Exp. 98 may occur in these direct-transmission experiments, it will be seen that in ten experiments the transmission was probably effected during the first application of the box. In some of the others, in which negative applications are recorded, it is possible that the incubation period may have been underestimated, and that the first application of the box was effective in these cases also.

It is, however, plain that in a considerable number of instances 35 hungry flies failed to transmit trypanosomes from sick to healthy monkey by the direct method. Exps. 60, 109, 135 and 161 failed altogether. In the three-fly experiment trypanosomes were very numerous in the blood of the infected monkey, and here the infection probably occurred on the first day.

As a general rule, though the point is not brought out in the Tables, the ease with which infection occurred depended on the number of trypanosomes in the peripheral blood. No doubt, as Roubaud has suggested, the success of direct-transmission experiments depends to a certain extent on the susceptibility of the clean animal to the trypanosome, and this factor must have contributed to Oehler's success with single parasites (Oehler, 1913). In the monkeys

Table IV.

Expt. No.	Size of monkey	Number of Lab.-bred flies employed in the transmission	No. of infecting monkey	Approx. day of disease in infecting monkey when transmission attempts began	Total number of days on which the interrupted feeds were performed from infected to healthy monkey	Dates on which routine application of flies for interrupted feeds failed to produce infection of healthy monkey	Date when trypanosomes first seen in blood of 2nd monkey	Passage no. in the series of direct trans-missions	Duration of disease in the 2nd monkey (in days)	Remarks
60	Medium	30	72	35th	18-19. iv. 20	Both days	—	—	—	No infection resulted
63	Large	30	43	35th	28. ii.-1. iii. 20, 11-17. iii. 20, 19. iii. 20, 22-26. iii. 20, 30. iii. 20	28. ii.-1. iii. 20, 11-17. iii. 20 and 19. iii. 20	3. iv. 20	1st	79	“nakabugo” species
72	Large	30	59	19th	10. iii. 20, 12-16. iii. 20, 19-20. iii. 20	—	21. iii. 20	1st	77	—
88	Large	30	72	16th	30. iii.-3. iv. 20, 5-8. iv. 20	30. iii.-3. iv. 20	21. iv. 20	2nd	—	—
93	Large	30 and 60	88	21st	29-30. iv. 20, 3. v. 20, 4-8. v. 20, 12-14. v. 20	29-30. iv. 20	15. v. 20	3rd	47	A Sesse monkey never very fit: an old animal
98	Medium	30	88	13th	21-22. iv. 20	—	4. v. 20	3rd	—	N.B. Incubation at least 13 days
107	Medium	30	98	14th	5-8. v. 20, 12-14. v. 20	—	15. v. 20	4th	140	—
109	Medium	5	72	61st	12-13. v. 20	Both days	—	—	—	No infection occurred
111	Medium	30	107	10th	15-19. v. 20	—	25. v. 20	5th	90	—
117	Large	30	111	10th	25-30. v. 20	—	3. vi. 20	6th	125	—
123	Medium	30	117	17th	11-15. vi. 20	—	22. vi. 20	7th	—	Ran away 2. vii. 20
124	Medium	30	59	120th	19. vi. 20, 21-23. vi. 20, 3-12. vii. 20	19 and 21-23. vi. 20	20. vii. 20	—	91	“Chronic disease.” Ran away 28. viii., recovered looking thin 8. ix. 20
132	Medium	30	123	17th	28. vi.-1. vii. 20	—	7. vii. 20	8th	254	—
140	Medium	30	132	10th	8-11. vii. 20	—	15. vii. 20	9th	94	—
147	Medium	50 and 30	140	7th	17-21. vii. 20, 23. vii. 20, 28-30. vii. 20, 2-4. viii. 20, 6-9. viii. 20	17-21. vii. 20 and 23. vii. 20	13. viii. 20	10th	90	—
161	Medium	30	147	19th	21. viii. 20, 23-28. viii. 20	21 and 23-28. viii. 20	—	—	—	No infection occurred
—	—	Inoculated subcutaneously on 28. ix. 20	—	57th	—	—	4. x. 20	11th	—	Monkey inoculated with 1 c.c. citrated blood of 147 and becomes 11th passage
160	Small	3	139	20th	13-17. viii. 20, 20-21. viii. 20, 23 and 25. viii. 20	—	27. viii. 20	—	—	139 showed T.'s +++ daily. On 20. viii. mosquito netting substituted for wire in fly box
164	Medium	30	160	17th	29. viii. 20, 31. viii.-1. ix. 20, 3-4. ix. 20, 6. ix. 20	—	15. ix. 20	—	—	—
135	Medium	35	117	40th	4 and 6. vii. 20	—	—	—	—	No infection occurred
121	Medium	30	127	35th	26. vii.-24. viii. 20, 26 and 28. viii. 20	All days	—	—	—	No infection resulted. T.'s varying from none in 10 mins. search in blood of 127 to 1 in 2 fields. Never numerous. M 127 was inoculated direct from infected native

used above trypanosomes were never very numerous, but often showed up to two or three per field with the $\frac{1}{6}$ th objective.

With the human strain all attempts at direct transmission failed completely. Trypanosomes were always rare in the blood, though on some of the days on which the feedings were carried out they were present up to one in two fields. Here, doubtless, the susceptibility factor comes in, as I have had the same experience as Bruce and others of the difficulty with which early passages of this strain are effected. Reference to Table I will show that there is no appreciable alteration of virulence as the result of the passages so far carried out.

Table VI shows that full cyclical development took place in laboratory-bred flies fed on Monkeys 88 (second passage, Exp. 48), 117 (sixth passage, Exp. 51), and 161 (eleventh passage, Exp. 85).

Conclusions.

(a) As far as the direct-transmission series has as yet progressed, *i.e.* to the eleventh passage, there is no sign of any loss on the part of the trypanosome of the capability to undergo cyclical development in the fly.

(b) Up to the present no evidence has come to light that the virulence of a strain is enhanced by continued passage by this method in the same species of mammal. It is, however, too soon to draw any conclusion on this point.

(c) Under natural conditions this method of transmission is likely to operate only when trypanosomes are present in fair numbers in the peripheral blood. Typical *T. gambiense* infections in man, as commonly recognised, do not, in the later stages at any rate, fulfil this requirement.

PART V. EXPERIMENTS WITH LABORATORY-BRED FLIES AND THE LAKE-SHORE AND HUMAN STRAINS.

The experiments presented in this section were devised to ascertain the extent to which the three strains of trypanosomes—the wild-fly, the antelope and the human strains—are transmissible by laboratory-bred *G. palpalis*; and further, to investigate the effect of different kinds of blood on these strains during their development in the fly.

The pupae from which the flies were hatched were obtained from the lake-shore in the neighbourhood of Entebbe. In some of the experiments notes were kept on the number of pupae produced under the different dietetic conditions, out of consideration for Roubaud's statement that mammalian blood is necessary for the reproductive processes of the tsetse (Roubaud, 1919). My experiments show, however, that full-formed larvae and pupae can be produced by flies fed solely upon *Varanus* blood, a result similar to that of Kleine with flies fed with crocodile blood (Kleine, 1909 and 1911 *a*). A whitish papular eruption, limited to the area bitten by the tsetse, signals the first few applications of flies to a new monkey; this rash disappears in a

few days. In the Tables, Nos. V, VI and VII, column 4 records the day upon which, after the first infecting feed, dissection was commenced. For the first few days of the experiment the dead flies are not dissected, as the discovery of trypanosomes in such early flies does not necessarily imply a developing infection. The dates appearing in the second column of the Tables are given to show the stage of the disease in the infecting mammal.

(a) *Meteorological conditions at Entebbe and Mpumu.*

Records are available of the wet and dry bulb readings inside the laboratory and at the Entebbe meteorological station in the open air.

The laboratory readings are on the whole higher than the official figures, and as compared to the figures obtained from Mpumu the Entebbe laboratory showed a generally higher temperature throughout the year.

(b) *Tables of fly experiments and discussion of same.*

The human, wild-fly and Damba antelope strains are dealt with in Tables V, VI and VII respectively. The percentage of infected and infective flies obtained with each strain is given at the end of the corresponding table. In none of the flies dissected were any flagellates found in the proboscis.

Tables V, VI and VII show that:

(a) With the limited number of flies employed, the average duration of the full developmental cycle, with the human strain, was 32 days (maximum 45, minimum 25 days); with the fly strain, average 29 days (maximum 31, minimum 26 days); with the buck strain, average 28 days (maximum 40, minimum 19 days).

(b) Fully formed and apparently healthy larvae can be produced by flies fed on a purely reptilian diet.

(c) All three strains of trypanosome can develop in flies which are nourished solely on reptilian blood. This matter will be further dealt with in subsection (d) below.

Discussion of the fly-dissection Tables.

It is much to be regretted that the salivary gland dissections were not more carefully performed in the experiments recorded in the above Tables. Press of other work made it impossible for me to undertake all the fly dissections myself, and the routine method practised by the native assistant consists in pulling out the gut, after snipping off the terminal part of the abdomen, and teasing it up in saline. In this way the glands usually come out with the intestines and are chopped up therewith, so that only fragments are available for scrutiny.

The destruction of two of the experimental boxes by ants was due to these boxes, after having been selected for dissection, being left on the table overnight instead of being replaced on the water dishes. The tiny ant responsible for these devastations appears with magical suddenness wherever anything

Table V. Transmission of the human strain of polymorphic trypanosomes, T. gambiense, by laboratory-bred G. palpalis.

Expt. No.	Infecting animal and date at which T.'s first seen in its blood	Period during which flies feed on infecting animal	Day after first infecting feed on which dissections commenced	Number of flies dissected				Whether or no the flies proved infective to clean monkey	Animal on which flies nourished during expt.	Remarks		
				♂	♀	Total	Showing flagellates in gut only				With flagellates in gut and salivary glands	
56	21. vi. 20	12-17. vii. 20	9	30	11	41	1 (12th day)	0	28	No	Monkey	M 127 infected by inoculation of patients' blood on 21. vi. 20 Glands of + fly nil From 17-18. viii. 20 interrupted feeds, only, on 127 From 20-30. viii. 20 interrupted feeds, only, on 127 2 pupae from 24th day, glands of + fly nil 16 pupae from 21st day 1 pupa on 33rd day. 17 flies alive on 25th day of experiment From 20th till 36th day 3 pupae and 1 immature larva: later 4 pupae. Glands nil of 41st day fly. 19 flies alive on 25th day of exp. Between 19th and 34th days 11 pupae. Throughout exp. 17 pupae. From 6-9. xii. 20 fed on Varanus. No + fly found during dissection. Box infective about 35th day. On 10. xii and 11. xii non-infective to clean monkey. Positive fly presumably lost. 39 flies alive on 25th day of experiment 1 small pupa on 23rd day. 39 flies alive on 25th day of experiment From 19th day 2 abortive larvae and 3 formed pupae. Glands nil, fragments seen. 70 flies alive on 25th day of experiment From 22nd to 33rd day, 4 pupae: subsequently 6 more pupae. 60 flies alive on 25th day of experiment
55	127	14-17. vii. 20	11	48	21	69	1 (11th day) 2 (30th day)	0	30	No	"	
65	127	14-21. viii. 20	10	12	14	26	0	0	53	No	"	
64	127	20. viii.-4. ix. 20	20	19	25	44	1 (10th day)	0	48	No	"	
{ 72 73 79	127	7-10. x. 20	10	19	23	42	1 (34th day)	0	34	—	Mangabey 86	
	127	7-10. x. 20	10	18	29	47	0	0	43	No	Monkey	
	127	17-19. x. 20	7	20	15	35	0	0	36	No	Varanus till 32nd day	
{ 80	127	17-19. x. 20	9	29	15	44	1 (25th day) 1 (41st day)	0	41	No	Monkey	
	127	23-25. x. 20	11	23	26	49	0	? 1	54	Yes	Monkey	
{ 87	127	23-25. x. 20	8	32	16	48	0	0	34	Not tried	Varanus throughout	
	127	25-27. x. 20	8	58	37	95	1 (33rd day)	0	33	"	"	
{ 88	127	25-27. x. 20	7	40	47	87	1 (7th day) 1 (8th day) 1 (50th day)	0	51	No	Monkey	
	127											

{93 94}	3. xi. 20 121 121	4-8. xi. 20	10	20	15	35	0	61	Not tried Yes	Varanus from 10. xi.-15. xii Monkey	From 31st day 2 pupae and 1 larva Flies devoured on 26th day by ants. Fly infective 25th day of experiment
		4-8. xi. 20	6	4	6	10	1 (6th day)	26			
		1-4. xii. 20	14	20	14	34	0	62			
		1-4. xii. 20	15	20	13	33	1 (15th day)	62			
		17-21. xii. 20	16	32	16	48	0	52			
		17-21. xii. 20	14	30	23	53	0	54			
		30. xii. 20-2. i. 21	10	35	21	56	2 (10th day) 1 (35th day)	57			
{98 99 100 101 106}	21. vi. 20 127 127	30. xii. 20-2. i. 21	13	24	11	35	0	57	—	"	Only small portion of distal end of glands of 35th day fly seen which negative. Fly in- fective 45th day of experiment
		1-5. i. 21	13	18	20	38	1 (23rd day)	44			
		1-5. i. 21	12	28	31	59	0	44			
{107 108 109}	3. xi. 20 121	—	17	59	35	94	1 (24th day) 1 (46th day)	46	Yes	"	On 44th day remaining flies, about 12 in number, were eaten up by ants Glands +++ in fly of 44th day. This fly had fed on 6-7. ii. 21 on baboon whose blood did not affect the T.'s as is shown by the fly infecting a clean monkey on 8. ii. 21 and 9. ii. 21. Fly infective on 29th day of experiment
Totals	—	—	—	629	484	1122	20 (18)* 3 (5)				Fly infective on 35th day of exp. One fly un- dissected on 31st day of exp. Glands of the 46th day fly very imperfectly dissected: portions seen contain few T.'s which how- ever may be gut forms which have pene- trated the broken-up bits of gland

Total percentage of flies *infected* with flagellates=2.0 % (allowing one each for Exps. 87 and 94, and including the infective flies)*.

Total percentage of *infective* flies=.44 % (allowing one each for each of Exps. 110, 106, 94, 87; in the case of Exps. 110 and 106 the infective flies are presumed to have been those dissected on 46th and 35th days respectively, the glands of which were improperly dissected).

Table VI. Transmission of the wild-fly strains of the polymorphic trypanosome by laboratory-bred *G. palpalis*.

Exp. No.	Infecting animal and date at which T.'s first seen in its blood	Period for which flies fed on infecting animal	Day after first infecting feed when dissections commenced	Number of flies dissected					♂	♀	Total	With flagellates in gut only		With flagellates in gut and glands		Strain of trypanosome	Duration of exp. in days	Whether or no the flies proved infective to clean monkey	Animal on which flies nourished during the experiment	Remarks
				1 (25th day)	1 (50th day)	With flagellates in gut only	With flagellates in gut and glands	1 (25th day)				1 (50th day)								
4	30. i. 20 43	2-10. ii. 20	28	14	11	25	1 (25th day)	1 (50th day)	Wild fly direct	67	Yes	Monkey	Subsequently infected a 2nd clean monkey with incubation period of 6 days. Fly infective on 19th day of experiment							
10	27. ii. 20 59	6-9. iii. 20 and 27, 29, 30. iii. 20	30	15	16	31	0	0	Do. (fly cycle off 43)	41	No	..	Between 9. iii. and 27. iii. fed on 59 by interrupted method only							
12	72	23. iii.-9. iv. 20	11	14	7	21	0	0	Do. (1st passage)	41	From 24. iii.-8. iv. 20 interrupted feeds only							
13	43	17-25. iii. 20	19	20	7	27	0	0	Do. (direct from wild-fly)	25	—							
14	21. iii. 20 72	21. iii.-8. iv. 20	16	9	9	18	1 (22nd day)	0	Do. (1st passage)	31	22. iii.-27. iii. and 30. iii.-8. iv. interrupted feeds only							
15	22. iii. 20 39	2-18. iv. 20	16	5	9	14	0	0	Do. (fly cycle off 43)	41	—							
16	39	5-18. iv. 20	16	7	6	13	0	0	Do. (fly cycle off 43)	39	—							
17	3. iv. 20 63	11-24. iv. 20	23	10	12	22	0	0	Do. (1st passage)	43	14 and 15. iv. interrupted feeds only							
18	63	11-24. iv. 20	35	12	11	23	0	0	Do. (fly cycle off 43)	42	—							
21	21. iv. 20 88	23-29. iv. 20	10	25	28	53	0	0	Do. (2nd passage)	31	—							
23	22. iii. 20 39	25. iv.-1. v. 20	13	11	30	41	0	0	Do. (fly cycle off 43)	46	—							
24	27. ii. 20 59	25. iv.-1. v. 20	22	16	17	33	0	? 1	Do. (fly cycle off 43)	56	Yes	..	{ No + fly found. Monkey fed on from 13. vi.-18. vi. not infected. Box fed 27. v. on mangabey and on 7-10. vi. on chimpanzee. Fly infective 26th day of experiment							
25	59	25. iv.-1. v. 20	16	12	9	21	0	0	Do. (fly cycle off 43)	55										
26	22. iii. 20 39	29. iv.-1. v. 20	11	12	28	40	0	0	Do. (3rd passage)	40	No	..	—							
31	4. v. 20 98	6-13. v. 20	22	20	26	46	1 (34th day)	0	Do. (3rd passage)	34	—							
34	27. ii. 20 59	15-24. v. 20	18	12	13	25	0	0	Do. (fly cycle off 43)	30	—							
35	15. v. 20 107	17-24. v. 20	16	14	23	37	0	0	Do. (4th passage)	28	..	Calf and monkey	—							

37	3. vi. 20 117	11-21. vi. 20	15	11	9	20	0	0	Do. (6th passage)	46	"	Monkey	11-13. vi. interrupted feeds only. Starved 14. vi.
38	117	14-19. vi. 20	23	13	18	31	0	0	"	44	"	"	—
39	117	16-19. vi. 20	28	18	16	34	0	0	"	43	"	"	—
40	27. ii. 20 59	19-24. vi. 20	21	18	17	35	0	0	Do. (fly cycle off 43)	45	"	"	—
42	21. iv. 20 88	19-24. vi. 20	18	12	15	27	0	0	Do. (2nd passage)	48	"	"	—
48	88	30. vi.-6. vii. 20	14	51	38	89	0	1 (38th day)	"	38	Yes	"	The + fly had a few T.'s in proximal part of gland only, indicating that the salivary gland infection was very newly established. Infective 31st day
51, 52, 53	3. vi. 20 117	3-10. vii. 20	17	48	39	87	0	? 1	Do. (6th passage)	42	"	" 12. vii.-7. viii.	Box 53 set aside for dissection after completion of 51 and 52 was entirely devoured by ants. Presumably the + fly was in this box
59	15. vii. 20 140	6-11. viii. 20	12	16	23	39	0	0	Do. (9th passage)	24	No	Monkey	—
58	21. iv. 20 88	6-11. viii. 20	11	26	14	40	0	0	Do. (2nd passage)	56	"	"	—
61	3. viii. 20 139	14-17. viii. 20	19	26	17	43	0	0	"	19	"	"	—
62	9. viii. 20 142	14-17. viii. 20	9	15	35	50	1 (9th day)	0	"	19	"	"	—
63	21. iv. 20 88	17-25. viii. 20	12	33	29	62	0	0	"	50	"	"	—
68	88	2-7. x. 20	13	18	23	41	0	0	"	34	"	Varanus	27 flies alive on 25th day of exp.
69	88	2-7. x. 20	15	16	21	37	1 (20th day)	0	"	34	"	Monkey	32 flies alive on 25th day of exp.
{ 74 75	27. ii. 20 59	9-12. x. 20	11	21	30	51	1 (30th day)	0	Do. (fly cycle off 43)	31	"	Mangabey 86	Mangabey is practically immune to the T.'s
	59	9-12. x. 20	9	20	25	45	0	0	"	28	"	Monkey	
85, 86	4. x. 20 161	21-24. x. 20	9	32	47	79	1 (9th day) 1 (51st day)	1 (51st day)	Do. (11th passage)	51	Yes	"	On 7, 8, 9. xii. fed Varanus. The + fly was infective on 31st day. Both 51 day + flies contained Varanus blood which apparently did not affect the flagellates
91	4. x. 20 161	29-31. x. 20	11	39	6	45	0	0	"	48	No	"	3 pupae from 22nd day
92	161	29-31. x. 20	10	28	6	34	0	0	"	48	"	"	3 pupae from 22nd day
96	161	Throughout exp.	11	41	19	60	1 (31st day) 1 (32nd day)	0	"	32	"	"	—
			Totals	730	709	1439	10	3 (5)					

Percentage of flies containing developing flagellates (including infective flies) allowing 1 for Exps. 24, 25 and 1 for Exps. 51-53 = 1.04 %
Percentage of infective flies (including 1 for Exps. 24, 25 and 1 for Exps. 51-53) ... = 0.34 %

Table VII. Transmission of the 1920 *Damba antelope* strain by laboratory-bred *G. palpalis*.

Experiment number	Infecting animal and date at which T.'s first seen in its blood	Period for which flies fed on infecting animal	Day after first infecting feed when dissections commenced	Number of flies dissected					♂	♀	Total	With flagellates in gut only	With flagellates in gut and glands	Strain of trypanosome	Duration of expt. in days	Whether flies proved infective to clean monkey	Animal on which flies nourished during experiment	Remarks
{70 71 77	{173 173 173	5-9. x. 20	11	22	18	40	1 (24th day) 1 (36th day)	0	Orig. monkey Damba buck	36	—	114	8 pupae from 23rd day					
		5-9. x. 20	10	16	18	34	2 (10th day)	0	„	38	No	Mangabey Monkey	4 pupae from 23rd day					
		13-16. x. 20	11	21	27	48	1 (27th day)	? 1	„	30	Yes	Monkey	45 flies alive on 25th day of exp. Infective on 21st day. From 24th day 2 pupae. Fragment of gland (distal) seen, nil					
{78	173	13-16. x. 20	11	26	33	59	1 (32nd day)	? 1	„	32	Yes	Varanus	47 flies alive on 25th day of exp. Box infective on 31st day which was first day it was tested. From 23rd day 1 aborted larva. Fragment of gland (distal) seen, nil					
{81 82	{173 17. x. 20	17-19. x. 20	10	21	20	41	0	0	„	24	—	112	—					
		17-19. x. 20	11	28	22	50	1 (39th day)	0	„	39	No	Mangabey Monkey	Fly only ++					
{83 84	{171 171	19-21. x. 20	8	31	22	53	1 (10th day)	0	Buck 1st passage	41	No	Monkey	7 pupae from 32nd day to 38th day. 37 flies alive on 25th day of experiment					
		19-21. x. 20	8	22	27	51	1 (34th day) 1 (36th day)	0	„	38	No	Varanus to 35th day	2 pupae from 22nd day. 34 flies alive on 25th day of exp.					
112	16. i. 21 227	21-24. i. 21	8	40	23	63	1 (18th day)	0	3rd passage	44	No	Monkey	—					
{113 116	{15. i. 21 Dog 232	24-26. i. 21	10	21	37	58	0	2 (31st day)	„	31	Yes	Monkey	} Fed on baboon (young) 20. ii. 21. ii: baboon did not become infected: fed clean monkey 22. ii and 23. ii which became infected. Glands swarming. 113 and 116 fed same monkey on alternate days					
		26-27. i. 21	7	18	39	57	1 (11th day)	0	„	32	—	Monkey						
114	16. i. 21 227	24-26. i. 21	10	27	35	62	0	1 (52nd day)	„	52	Yes	Monkey	} Fly infective on 40th day of exp. Infected in succession on clean monkey, a clean sheep and a clean monkey. Glands ++. 114 fed same monkey on alternate days					
115	227	26-27. i. 21	12	27	26	53	1 (51st day)	0	„	51	—	Monkey						
118	227	26-27. i. 21	8	36	28	64	1 (20th day)	0	„	61	No	Monkey						
Totals				356	375	731	14 (12)	3 (5)										

Percentage of flies containing developing flagellates=2.32.

Percentage of infective flies (allowing one each for Exps. 77 and 78—probably the flies dissected on 27th and 32nd days of the respective experiments, cf. above)=0.68.

suitable for food is left unprotected, and the following morning some wing fragments and a few wandering loiterers are all that remain to mark the tragedy.

In view of these sources of error there is nothing in the above experiments inconsistent with the generally accepted belief that, with this trypanosome, a fly is infective when its glands are invaded, and not before. The period that elapsed after the first infecting feed before the flies became infective, in each of the five positive experiments of Table V was 35, 25, 45, 29 and 35 days respectively. Miss Robertson found in the course of her investigations into the development of trypanosome strains in *G. palpalis* at Mpumu, that in one case the glands became infected on the 12th day, and in rare cases on the 16th day, gland infections being increasingly frequent after the 20th day. She states that a fly is capable, apparently, of conveying infection when only the proximal part of the gland, close to the duct, shows parasites (Robertson, 1913). It is thus easily possible to overlook a gland infection when only fragments of the distal end of the viscus are inspected. Under the coverslip, a positive gland may often be seen almost completely to empty itself of trypanosomes through a hole in its side caused by pressure.

Miss Robertson also refers to the rather rare cases where a fly may show a considerable number of flagellates in the gut as late as the 56th day of the experiment, without the salivary glands being infected. She found that, under ordinary feeding conditions (as contrasted with certain starvation experiments) the strains of trypanosomes employed produced 3 per cent. of infected flies. Kleine and Fisher, in experiments with *palpalis* and *T. gambiense* at Rutschugi, a region free from Sleeping Sickness, found that 8 of their 881 experimental flies became infective, *i.e.* 0.9 per cent.; and that of the flies that lived for more than 12 days, slightly more than 1.7 per cent. contained flagellates; the cycle lasted 28–31 days. In a sleeping sickness region 2.5 per cent. to 6 per cent. of their flies became infective, and the cycle lasted 20–25 days (Kleine, 1913).

Bruce and his co-workers at Mpumu gave 27 days as the shortest time in which a fly became infective with *T. gambiense*, the longest cycle taking 53 days, and the average 36 days (Bruce, 1910 *a*).

In Tables V, VI, with both the human and wild-fly strains, the percentage of infected flies is lower than that obtained at Mpumu. This is curious, as one would expect that conditions at the Entebbe Laboratory a few feet above lake level would be more favourable than at 600 feet higher on the top of the Kyagwe hill. As a rule, in the present experiments, the flies were only fed on the nourishing animal every other day, while those at Mpumu were fed daily except on Sundays. Again at Mpumu, in all save Miss Robertson's experiments, cocks were employed to a considerable extent in nourishing the flies during the development of the cycle. In individual experiments at Mpumu the percentage of positive flies reached as high as 20 per cent. (Duke, 1913 *b*).

(c) *Failure to find flagellates in flies held to be infective.*

An important point in experiments with laboratory-bred flies is whether an infective fly can, under any circumstances, become cleaned of its flagellates, either in gut or glands or both. Miss Robertson obtained no evidence that a trypanosome infection once established in a fly is ever got rid of, meaning, of course, under normal feeding conditions. Experiments at Mpumu on the effect of arsenic-containing blood on positive flies, showed that flies fed on a monkey sufficiently soon after the dose of arsenic lost their gut infection while the trypanosomes of the glands were unaffected (Duke, 1913 *a*). Similar results were obtained by Roubaud with proboscis-and-gut infections.

Bruce and his co-workers at Mpumu and in Nyasaland record two interesting observations. Twelve *palpalis* remaining in positive Exp. 975 were dissected and found negative to flagellates; they were then pooled and injected into a healthy monkey which became infected. The inference drawn from this experiment was that an infective fly may escape detection by the microscope (Bruce, 1910 *b*).

The Nyasaland instance is that of a positive experiment of *morsitans* in which an infective fly had been isolated in a glass tube and had, alone, infected a mouse and a rabbit. The fly remained alive in the tube for 13 days, and on dissection proved to be free from trypanosomes throughout: another example of the same kind was subsequently observed. The inference drawn was that it must, therefore, be held as probable that an infective fly, with presumably both salivary glands and alimentary tract swarming with trypanosomes, can lose all these flagellates and become non-infective (Bruce, 1914 *a*).

It was thought possible that by feeding positive flies upon animals which are naturally resistant or immune to infection by the trypanosome, a complete or partial clearance of the fly might be effected. That this is not the case with baboon blood and either the human or the buck strain, was shown by the presence of flagellates swarming in both gut and salivary glands of the positive flies of Exps. 109 (human) and 113 (buck), both of which boxes had fed well for two successive days on a baboon. In Exps. 85 and 86, also, the positive fly contained quantities of *Varanus* corpuscles, but the flagellates in both glands and gut were unaffected.

When confronted by the absence of flagellates from flies which are presumed to have infected a clean animal, the first thought should be that a contamination infection of the clean animal has occurred, as, for instance, the direct transmission by wild biting flies from a neighbouring infected animal. A second possibility is that the gut has by some means been cleaned, as happened in the arsenic experiments above referred to, the glands remaining infective.

These two contingencies must be excluded before an explanation is sought in some obscure and hitherto unsuspected process of sterilisation, or in the more simple conclusion that the positive fly has been overlooked.

As, under ordinary circumstances, the glands of flies in my experiments were not dissected out unless flagellates were seen in the gut, it is possible that a fly with positive glands and negative gut, if such occurred, would escape notice. This is, however, not probable.

The possibility of natural contaminating infections has been most carefully investigated. In addition to control monkeys kept for months under daily examination, a particularly severe test was devised to settle this point. Two baby monkeys, brought in with their mothers and still unweaned, were allowed the freedom of the monkey village. They roamed about all day long among their friends, and were examined daily. The mother of one was infected with the relatively virulent buck strain. Both these babies spend much of their time in the boxes of infected monkeys near by, and sit hugged up close for long periods at a time. They have been under observation for months, and neither they nor the other controls have ever become infected. I have never seen an instance of a natural infection among the monkeys at this laboratory. I think, therefore, that it is justifiable to assume that the infective flies in those positive experiments, such as No. 87, in which no positive flies were found on dissection, were overlooked during the removal of the daily "deads" until they had dried up; and this may well happen, unless special care is taken, owing to the tendency of dead flies to become caught up in the corners of the boxes.

(d) *Effect of different kinds of blood on the developing flagellates*
(as revealed by Tables V, VI and VII).

Lizard. Kleine at one time held the view that monkey's, and more especially man's blood, was more favourable than that of ruminants to the development of trypanosomes in the fly (Kleine 1911 *b*). Subsequently he gave up this opinion and expressed himself as convinced that the type of blood taken up by the fly was a wholly unimportant factor (Kleine, 1913). At Mpumu a single series of experiments (Duke, 1913 *b*) pointed to yet a different conclusion, namely that ruminant blood was more favourable than monkey blood; the percentage of infected flies in the experiments were: on ruminant blood 9.1 per cent.; on monkey blood 2.1 per cent. In the experiments recorded in the present paper an attempt was made to pursue this matter further and, keeping in view practical issues, to discover whether the various strains of trypanosomes employed were capable of developing in flies fed for a prolonged period on reptile blood.

Observers who have attempted to feed laboratory-bred flies on crocodiles have been struck with the difficulty experienced in persuading the flies to feed. As no crocodiles were available, water-lizards (*Varanus* sp.) were employed, since they are easily obtained and live for months in captivity on a diet of shell-fish and crabs.

At first the greatest difficulty was experienced in making the flies feed. One would feed, and then perhaps one or two more, but often three-quarters

of an hour would go by and only few of the insects take their fill. That they were hungry was obvious by their efforts to bite the fingers of the operator; directly they were placed on the reptile, however, they showed no further interest. Briefly, after much experimenting, the flies were found to feed well when housed in mosquito-net instead of wire-sided boxes, and placed on the lizard after exposure of the latter to the sun for a short time. The reptile proved extremely sensitive to sun heat, soon becoming very distressed, with protruded tongue and open mouth, and gasping for breath. The box of flies was placed on the reptile's flank, and the upper netted side covered with a wet cloth to shut off the distracting effect of the operator's hand. Now and then a cautious peep under the cloth revealed whether or not the flies were feeding, and if none were so engaged the box was lifted away and replaced again, if necessary gently stroking the animal's skin under some conveniently situated empty fly to stimulate the insect to start feeding. With proper care and patience almost every fly will feed, and, with increasing familiarity, feeding becomes more rapid. The flies take as a rule longer to finish their feed on the lizard than on a monkey, the first part of the sucking act seeming the most difficult. It was noticed that once the flies in a box had fed well on the lizard, it was little or no use trying to feed them on the following day, whereas on monkeys the flies will feed readily day after day. Possibly the nuclear material present in the *Varanus* corpuscles accounts for this difference.

Full-grown lizards do not appear to take the slightest notice of the flies, even when two boxes of flies are fed simultaneously on either flank; the first lizard employed, however, was a young animal, and it became increasingly restive until the insertion of a proboscis resulted in a general upheaval which eventually made feeding impossible.

No trypanosomes were ever detected in the lizards employed in these experiments, nor were forms resembling *T. grayi* ever seen in the various flies fed upon them; dissection of 159 clean laboratory-bred *palpalis* fed for 33 days on the experimental lizards failed to reveal any developmental flagellates.

Reference to the pairs of parallel experiments in Tables V–VII will show that, although there is, perhaps, some indication that the number of flies containing flagellates is less on a diet of *Varanus*, than of monkey blood, yet it is possible, with the buck strain at any rate, for a fly to become infective on such a diet.

For the paired experiments—devised so that the conditions of infective feeding were identical and the subsequent diet of the flies, reptile, or monkey blood respectively—the figures are:

<i>Varanus</i> ...	Total flies dissected after the 7th day	...	364
	Containing flagellates	4 (1.09 %).
Monkey ...	Total flies dissected after the 6th day	...	328
	Containing flagellates	10 (3.04 %).

In the total of infected flies nourished on monkey blood in these parallel experiments the infective fly of Exp. 94 is not included.

Taking by themselves the positive flies in the paired experiments with the *human strain* we have:

on reptile blood:	Total flies dissected	... 213
	Containing flagellates	... 1;
on monkey blood:	Total flies dissected	... 190
	Containing flagellates	... 7;

i.e. 0.46 per cent. as against 3.68 per cent. infected flies on reptile and monkey blood respectively.

Mangabey sp.:

Mangabey Number of flies dissected after the 19th day	... 134
	Flies containing flagellates 2
Ordinary species Number of flies dissected after 9th day	... 142
	Flies containing flagellates 1

These dissections, limited though they are, indicate that a continued diet of blood of this resistant species does not harm the developing flagellates.

The number of flies used in these experiments is unfortunately small; it is hoped to continue investigations on these lines on my return from leave.

(e) *Conclusions as to the effect of reptile blood on developing trypanosomes.*

As far as conclusions are warranted by the limited evidence it seems that reptile blood exerts a relatively unfavourable influence on polymorphic trypanosomes developing in laboratory-bred *G. palpalis*. This unfavourable effect is especially noticeable in the case of the human strain of trypanosomes. Flies can, however, become infected and infective on such a diet.

PART VI. GENERAL REVIEW AND SUMMARY.

A consideration of the above experiments, and of the available literature dealing with the distribution of trypanosomes and tsetse flies in nature, has suggested certain general conclusions which will now be put forward.

(1) *Glossina palpalis* is essentially not a game tsetse in the sense that *G. morsitans* and *G. pallidipes* are. Its main food-animals, under primitive African conditions, are reptiles, possibly birds, man and his stock, the hippopotamus, and such game animals as may visit its haunts.

(2) In inhabited regions, wherever the wild *G. palpalis* are known to carry a polymorphic trypanosome with "anterior station" in the salivary glands, cases of trypanosomiasis of the *gambiense* type will generally be found to occur in man. The polymorphic trypanosomes so far recovered from game, stock, and man in inhabited *palpalis* areas, fall into line rather with *T. gambiense*

than with *T. brucei*, as regards virulence in laboratory animals and the absence of posterior-nuclear forms.

(3) With one certain and one doubtful exception, wherever the *brucei* type of trypanosome has been recovered from game or stock in a *palpalis* area, there have always been other tsetse species present in that area, and investigation has often actually proved that it is the other species that transmit this trypanosome in nature. From the foregoing considerations—which must be taken in conjunction with what has previously been said regarding “species” (see Introduction)—it seems reasonable to conclude that *the relatively avirulent polymorphic trypanosomes of palpalis areas, be they found in man, stock, or game, belong to a single natural species. This species is capable, on occasion, of parasitising man, and when it does so, the trypanosomes found in his blood are those which have for years been called T. gambiense.*

(4) Now the only two instances, where a trypanosome possessing the characters of the *brucei* group has been recovered from a pure *palpalis* region, occur in areas which have for years been uninhabited by man and where antelope abound, namely the Sesse Islands and Kiraro Lake near Lake George. There is, apparently, no region in Africa where antelope play such an important part in the dietary of *G. palpalis* as on the Sesse Islands at the present day, and where, in consequence, the polymorphic trypanosomes carried by this fly have an antelope as their almost exclusive host (the hippopotamus has been shown by Carpenter to be much less patronised by this fly than the situtunga, where both are available as food animals).

(5) In the old days the situtunga must have obtained their mammalian trypanosomes from *G. palpalis*; leeches and ticks do not appear to have played any part in the transmission of these parasites. But let us consider the distribution of trypanosomes on these islands to-day. In neither the wild fly nor the antelope have trypanosomes of the *nanum-pecorum* group ever been detected, and these organisms showed great reluctance to undergo full cyclical development in laboratory-bred flies at Mpumu. On the other hand *T. vivax* and *T. uniforme* are common in both buck and fly. In addition, we find in both buck and fly the polymorphic organism described from Damba.

(6) Whether the Sleeping Sickness epidemic was started by the entry of *T. gambiense* from some neighbouring endemic area or was due to an already existent organism whose spread was favoured by the terrible famine in Busoga, it is certain that a polymorphic trypanosome described as *T. gambiense* was present in the blood of an enormous number of human beings from 1901 to 1909 in the Uganda Lake *palpalis* areas.

(7) The part played by direct transmission in the spread of this epidemic we shall never know. It is certain, however, that a relatively large percentage of the wild fly must have been cyclically infected with the human trypanosome.

(8) With the gradual depopulation of the area by removals and by death, the cattle—the majority of which did not leave the islands until the final

exodus—the antelope, and the hippopotamus would be increasingly drawn upon by the fly for mammalian blood.

(9) We know that *T. gambiense* will survive for many months in the blood of the situtunga, and the antelopes must have become infected with the human trypanosome as the departure of man brought them into ever-increasing contact with the tsetse. From 1909 onwards, *Tragelaphus spekei* and the hippopotamus have constituted the mammalian food supply of the fly, and *ipso facto*, the reservoir of its trypanosomes.

(10) At the present day antelope swarm on some of the larger and a few of the smaller islands, and have extended their range since 1912 to islands on which, at that time and previously, they were unknown. Their numbers are enormous and their relation to the fly is very intimate. In 1912 the wild fly and the situtunga were still carrying a polymorphic trypanosome whose general behaviour was that of *T. gambiense*, and *there appears to be no reason to doubt that the polymorphic trypanosomes in the situtunga and wild G. palpalis in the prohibited area of Lake Victoria at the present day are the descendants of the T. gambiense which occurred in man at the time of the epidemic.*

(11) At the present day certain of these wild strains differ to a greater or less extent from the 1912 types; but as far as is known, there has been, since 1909, no introduction of fresh strains from outside, so that this cannot be the explanation of these differences. In the last few months evidence has been acquired of the occurrence of significant variations in the virulence and morphology of the trypanosomes on Damba, the island where the relations between fly and buck are more intimate, probably, than anywhere else. The variations observed tend towards the assumption of the characters typical of *T. brucei*.

(12) *T. brucei*, when it occurs in a fly-infested game region, is, as a rule, readily recoverable from both game and fly. Had such a trypanosome existed in the lake-shore *palpalis* area so thoroughly investigated by various observers in Uganda, it seems incredible that its presence should not have been detected by 1912. As stated above, trypanosomes of this type have not, as far as I can ascertain, been recorded from any purely *palpalis* area outside Uganda Protectorate.

(13) The difference between the only two strains so far recovered from Sesse situtunga is clearly marked. On the other hand, it must be admitted that, until the recovery of this second buck strain, no particular care was taken to search for posterior-nuclear forms in the strains obtained from lake-shore flies. These strains all produced more or less chronic infections in monkeys, but relatively few sub-inoculations were made into small animals. *As regards the 1912 antelope strain, however, it is quite certain that posterior-nuclear forms did not occur*, as this trypanosome was carefully examined at the time to see whether it showed any points of resemblance to the *brucei* type (Duke, 1912 *b*).

(14) While it is thus *possible* that posterior-nuclear forms occurred in

the 1912 fly-strains and were overlooked, it must be remembered that attention was first drawn to the peculiarity of the 1920 buck strain by its movements and by the way in which it killed its monkey host, and that posterior-nuclear forms were at once seen in the first guinea-pig inoculated. Years of experience have made me familiar with the behaviour of the local green *Cercopithecus* in captivity, but the disease in this animal was something I had not seen before.

(15) It is highly improbable that the newly recognised strains of trypanosomes could have been introduced by such poachers as have visited the islands since the depopulation. These people go to fish, and do not take dogs or stock with them. *It appears reasonable, therefore, to regard this newly-recognised brucei-like organism as a strain or variety of the older and formerly more common trypanosome whose general behaviour so strongly resembled the human parasite, T. gambiense.*

(16) The situation presented by the uninhabited fly-area in Uganda is unparalleled in Africa, and amounts to a demonstration, on a colossal scale, of the effects of environment on a strain of trypanosome. Before the epidemic, when the islands were teeming with natives and stock, the main food-animals of the *G. palpalis* were undoubtedly reptiles and man and his domestic animals. Reliable native accounts agree that the situtunga were comparatively rare in the palmy days of Sesse, and were seldom seen save on the extreme southernmost islets, whither great parties went at intervals to hunt them. The antelope may thus be regarded as an almost negligible source of food to the fly before the epidemic. The revolutionary change in the economy of the fly, effected by the sudden wholesale removal of man from its reach, has, with the passage of years, resulted in the usurpation by the antelope of the position formerly occupied by man and his stock. Among the reptiles, as Carpenter has pointed out, the result of the change has probably been a great increase in *Varanus*, more or less at the expense of the crocodile; for the natives prized *Varanus* skins very highly for their drums, and this lizard is very fond of crocodile eggs.

(17) Whether man or his stock or the antelope carried polymorphic trypanosomes in the days before the epidemic we shall, unfortunately, never know. We must, however, admit that the occurrence of human trypanosomiasis in the Uganda Lake area before 1899–1900, however probable it may be in theory, is not supported by native evidence. At all events, the existence of a human disease presenting the symptoms of the “mongota” of the epidemic is denied alike by the Baganda and the Basoga and the Basesse.

(18) It appears significant that the most virulent strain of trypanosome so far isolated was recovered from Damba Island, for on this island the fly and antelope are in more intimate relation than anywhere else. Both are very numerous, and the topography of the island entails constant exposure of the animals to fly-bite. Here, too, the effects of the close feeding of ever-increasing numbers of antelope are not yet pronounced. As Fiske was the

first to appreciate, many of the old fly-shores on Bugalla Island have been practically cleared of tsetse by the removal of suitable shelter all along the lake edge by the hungry antelopes. Branches are cropped off to within four or five feet of the ground, and all undergrowth is grazed off short. This denudation is also occurring on Damba, but has as yet had little effect on the density of the fly.

(19) It remains to be seen whether, as time goes on, the *brucei*-like organism will become increasingly common. At present the main differences between the Damba-antelope trypanosome and the other lake-shore strains are the disease which it produces in monkeys, and its greater virulence to other laboratory animals. Posterior-nuclear forms are also more commonly met with than in the mainland and island fly-strains. Damba Island will not be interfered with during the progress of the repopulation scheme, as I have especially requested that it be left undisturbed, in order to test this point. In the absence of leopards, crocodiles and pythons are the only enemies of the situtunga on this island, and the animals will presumably continue to multiply until either disease or failure of food checks their increase. Migration to the neighbouring island of Kome has occurred and will continue. It is possible that the animals will multiply to such an extent that direct transmission of their trypanosomes will become possible and will actually occur, with consequent increase in virulence for this now tolerant host, and the production of an epizootic of trypanosomiasis.

(20) *The practical importance of this discussion lies in the answer to the question as to whether or not, simultaneously with the acquisition of these minor changes, these strains may have lost their capability of survival in man.* It is possible that, in regions where man plays a big rôle in the fly's food supply, his removal and the substitution of another mammal—the situtunga—in his place may, in the course of years, lead to changes in the constitution of the trypanosome. It is easily conceivable that those species of trypanosomes which possess the power of utilising man as a host may lose this power if maintained for sufficiently long periods under natural conditions exclusively in ruminants.

(21) We have at present, under Mr Fiske's scheme for repopulation of the islands on sanitary principles, a considerable number of natives exposed to fly bite. In the course of the preliminary clearing, which is an important part of the scheme, frequent exposure is unavoidable. Furthermore, in the past few years a number of canoemen have been extensively exposed to fly in areas where the *gambiense*-like organism occurs in buck and fly. So far no cases of trypanosomiasis have occurred, but the experiment—for such it is—is still too recent to be conclusive.

(22) It may be contended that these wild-fly and antelope trypanosomes are not and never have been *T. gambiense*, but belong to some species proper to antelope and as yet unnamed. This contention is irrefutable by direct experiment, but the great bulk of the evidence indicates that we are dealing

with the descendants of the trypanosome which was parasitic in man at the time of the epidemic. A careful watch will be kept over the reinstated natives, and more especially over the fishermen, who will in this case also provide a natural experiment on a large scale.

(23) The reintroduction of man into the fly area, without careful precautions against re-establishing that close contact between man and insect which prevailed in the old days, would, in my opinion, lead eventually to the reappearance of human trypanosomiasis. Under the present scheme, however, the contact between man and fly is reduced to such an extent that, even if the existing organism is still capable of surviving in man, there will be no chance of the disease spreading. On the Mpologoma the disease still occurs endemically, and it is no great distance from this centre to the great lake; but every effort will be made to avoid the introduction of a fresh supply of human trypanosomes into the repopulated area. The scheme for carefully controlled repopulation of the fly zone will not fall to the ground even if sporadic cases of trypanosomiasis occur among the returned populations. The contention is that, with proper organisation, these areas can once again play their part in the economy of the Protectorate, without the mortality from this cause amounting to a serious figure.

(24) The indication recently forthcoming from Damba, that certain of the wild trypanosomes are developing increased virulence in laboratory animals suggests a far-reaching train of thought concerning the phylogeny of this group of organisms. As has been pointed out above, the Sesse Islands present a picture unparalleled in fly bionomics in Africa. *G. palpalis* is here in the closest contact with an antelope, and is undoubtedly feeding very freely on this mammal in spite of the existence of reptilian hosts. The chief mammalian host of the polymorphic trypanosomes of Damba at the present day is undoubtedly the situtunga, just as the chief hosts of these trypanosomes in *morsitans* areas are the game animals. The present close relations between fly, trypanosome, and antelope on Damba have steadily developed since 1909, and, at the present time, are closely parallel to those obtaining in typical big-game and *morsitans* country, always excepting the presence of reptiles. *It is possible, therefore, that the prolonged sojourn of a polymorphic trypanosome exclusively in game animals through many generations may result, per se, in the assumption by the parasite of the characteristics of the trypanosome known as T. brucei, irrespective of the tsetse species concerned with its transmission.*

(25) The foregoing considerations suggest that the differences between *T. gambiense* and *T. brucei* depend upon the predominance in *T. gambiense* areas of hosts other than ruminant game, *i.e.* man and possibly the hippopotamus and reptiles also. On this assumption is explained the failure to recognise the *brucei* type of trypanosome in pure *palpalis* areas elsewhere in Africa; for nowhere else have extensive observations been made in an area—if indeed such exists—where the removal of man has led to *G. palpalis* becoming closely and almost exclusively associated with antelope as a source of

mammalian blood. The occurrence in *brucei* regions of *gambiense*-like trypanosomes such as *T. multiforme* will also be intelligible when we bear in mind the opportunities which occur for the introduction of the *gambiense* type of organism in its human host into *morsitans* country, and the time which must elapse before the change of vertebrate environment can make itself felt on the trypanosome. The evidence from *morsitans* areas seems to be that, until recent years, man has been immune to the locally widespread *T. brucei*, and is still so in some areas. Similarly, negative evidence in Uganda points to the conclusion that the exposure of natives, at the present day, to the *palpalis* of the lake-shore prohibited area, does not result in man becoming infected every time that he is bitten by a cyclically-infected fly. The introduction of a number of human beings into the fly area might well lead, however, to the gradual acquisition by the trypanosome of powers to survive in these hosts. This seems to have happened in Rhodesia and in Nyasaland, and the same thing may happen on Sesse unless special precautions are taken to prevent it.

(26) There is another line of speculation which, however grotesque it may appear to those who believe in a multiplicity of species among the polymorphic trypanosomes, at any rate suggests a line of practical investigation to which the literature at my disposal makes no reference. The polymorphic mammalian trypanosomes are parasites for whose propagation in nature a long sojourn in the blood-host (mammal), with consequent maximum exposure to the insect intermediary, is desirable—if not essential. Trypanosomes tend to become adapted to their blood-hosts in such a way that they assume the character of harmless guests rather than pathogenic parasites. An acute and fatal disease of the host is detrimental to the parasite. Those mammals, therefore, which display true tolerance to a particular trypanosome may be regarded as its natural hosts.

For the main groups of pathogenic African trypanosomes the *tolerant* hosts are as follows:

- (1) *T. vivax* group game, and possibly stock.
- (2) *T. nanum* group game, and possibly stock (cf. below).
- (3) Polymorphic group:
 - (a) capable of survival in
 - man—*T. gambiense* ... man (in endemic areas) stock and game.
 - (b) incapable of survival in
 - man—*T. brucei* ... game, and possibly stock.

Man and game are antagonistic to some extent, for big-game country is not suited to extensive human occupation; that is to say, large numbers of game animals and large native populations are, under primitive conditions, mutually exclusive.

(27) The two principal game tsetses, *G. morsitans* and *G. pallidipes*, inhabit as a rule country where man would play but a minor part as a food animal. On the other hand, man, in primitive Africa, was probably often

associated with *G. palpalis*, in the dietary of which fly he still plays an important part in many districts. To-day the polymorphic trypanosomes of *palpalis* areas are of comparatively low virulence in the primitive food mammals of such regions—man and his stock and such game as may be present. Reptiles are, as far as is known, immune under natural conditions; and the rarity of trypanosomes in the large number of hippopotami examined by various observers suggests that this animal is in a marked degree tolerant, if not practically immune. These *palpalis*-trypanosome infections run a more or less chronic course in laboratory animals, and the parasites themselves show no posterior-nuclear forms in so far as they have been examined in this respect. Game-tsetse or *morsitans* areas are characterised, on the other hand, by polymorphic trypanosomes of the *brucei* type, to which, it appears, man has been immune until recent years—the tolerant hosts being essentially the game. Stock may be reckoned as relatively susceptible, since natives in many parts of Africa have from time immemorial avoided grazing their cattle in the proximity of big game. The *morsitans* trypanosomes cause an acute infection in laboratory animals, and tend to show posterior-nuclear forms. With one exception—*T. pecaui* of Roubaud—all the polymorphic organisms whose development in *Glossinae* has been studied, have their “anterior station” in the salivary glands of the fly.

(28) Let us for a moment suppose that, instead of a number of species of polymorphic trypanosomes differing from one another according to biometrical, serological, or laboratory pathogenicity tests, *there existed originally, throughout the tsetse areas of primitive Africa, a single species, characterised by polymorphism, and by its development in the salivary glands of Glossinae* the mammalian hosts of this organism would differ, we may suppose, in different regions—according to the food proclivities of the prevailing tsetse species. In *palpalis* areas, under primitive African conditions, the fly would feed mainly on man and his domestic animals, and on game, where it was available. In *morsitans* and *pallidipes* areas, under similar undisturbed conditions game would be selected. In each type of fly-area, natural selection would tend to establish tolerance to the trypanosomes on the part of those mammals which constituted the main food supply of the particular tsetse concerned with their transmission in that area. Introduction of new mammals into the food regimen of the tsetse might react in three different ways on the trypanosome: (a) the parasite might be unable to survive in the new host, in which case it would die out; (b) it might give rise to an acute and rapidly fatal disease, in which case it would automatically become extinct; or (c) it might find the new host suited to its requirements—i.e. tolerant—in which case it would survive and become established.

(29) Hitherto we have considered chiefly the effects produced upon the trypanosome by sojourn in different vertebrate hosts. But in parasites such as these, with a life-history normally passed in two different hosts—vertebrate and invertebrate—we must also take into account the possible effects

produced by sojourn in different species of *Glossina*. (It is conceivable, further, as Bagshawe has suggested, that climate may have some effect upon the physiological characters of the parasites; but at present this factor is too problematic to be discussed with profit.) As regards the influence exercised by the fly, we must note the following points:

(30) We know that *T. gambiense* is transmissible by *G. morsitans* and *T. brucei* by *G. palpalis*. What we do not know is whether, after a suitable number of experimental passages through the normally foreign tsetse species, *T. gambiense* acquires increased and *T. brucei* diminished virulence to laboratory animals; in other words whether the differences between *T. gambiense* and *T. brucei* are determined by the species of tsetse concerned with the transmission.

(31) The matter is obviously susceptible of direct experimental proof, but before a decision is reached a considerable number of passages must be made. The only experimental evidence that I can find consists in (a) some experiments by the Nyasaland Commission with a Tanganyika strain of *T. gambiense* and *G. morsitans*; (b) the transmission of the Mpumu laboratory strain of *T. brucei* by *G. palpalis*; and (c) of an East African organism by the same tsetse. All these experiments are too limited to justify any definite conclusion.

(a) In Nyasaland the *gambiense* strain was cyclically transmitted from monkey to monkey by laboratory-bred *G. morsitans*. The particular strain employed was inoculated by the syringe into nine other monkeys, of which two never became infected, two were alive on the 373rd day, two were alive on the 285th day; and, of the three that died, the maximum, minimum, and average duration of the disease was 264, 217 and 253 days respectively. The two monkeys infected by laboratory-bred *G. morsitans* died in 46 and 217 days. Two other Tanganyika *gambiense* strains were inoculated by the syringe into monkeys. In all, eight animals were employed of which four never became infected, two were alive on the 275th day and one on the 373rd day. The duration of the disease in the one that died was 31 days. The incubation period in this animal, before trypanosomes appeared in the blood, was 23 days, suggesting a chronic type of infection, so that the death of the monkey eight days later was perhaps due to some other cause. The same objection applies, however, to the brief course of the disease in one of the fly-infected monkeys (Bruce, 1915).

(b) The experiments in which *T. brucei* was transmitted by *G. palpalis* were only three in number, and as far as they go point to no attenuation of virulence in the first passage (Fraser, 1912).

(c) With the *brucei*-like organism from East Africa, also, no attenuation was noticed in the first generation (Duke, 1913 c).

The German investigators do not state the duration of the disease in the monkeys of their experiments, and no attempt was made to transmit beyond the first generation.

(32) From the foregoing considerations it may therefore be concluded that there is at present no certain evidence to prove that the invertebrate host determines—or influences—the characters of the trypanosomes which it is capable of transmitting; and the evidence supplied by the discovery of a virulent strain of polymorphic trypanosomes on Damba is against such a supposition. Whether the species of tsetse is a crucial factor in the production of the *brucei* or *gambiense* type can, however, be put to the test of experiment.

(33) The arguments briefly developed in the preceding paragraphs have, no doubt, presented themselves to other investigators of trypanosomiasis in Africa. They are not advanced here as incontrovertible truths, but rather as a legitimate working hypothesis which may serve as an incentive to further useful research. But the point that I wish to emphasise is that, in any strain of trypanosomes, the physiological characters—including ability to infect man—are largely determined by the environment. If the arguments here advanced are sound, then we are justified in concluding that *the polymorphic mammalian trypanosomes of Africa all belong to a single species, and not to a multitude of species*. This species is characterised by its *polymorphism in its vertebrate hosts, and its anterior station in the salivary glands of its Glossina intermediary*. Included in this species there are many different *varieties or strains, distinguishable from one another by characters of minor systematic importance*. *These strains are not immutable, but variable, and are determined by the environment in which the species lives: they are not constant, under varying external conditions, but each different strain is dependent upon, and is produced as a response to, a particular environment*. If we call this species *Trypanosoma brucei*, then “*T. gambiense*,” “*T. rhodesiense*,” and “*T. nigeriense*” are to be regarded as particular strains of *T. brucei* which have become, after sojourn in other hosts, more or less adapted to life in the blood of man.

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NOTES BEARING ON. LEEUWENHOEK, REDI, LIN-
 NAEUS, DE GEER, RUDOLPHI, MÜLLER, LEIDY, OWEN,
 JENNER, KIRCHER AND BOYLE, WHOSE PORTRAITS
 HAVE APPEARED IN *PARASITOLOGY*, VOL. XIII.
 (PORTRAIT-PLATES I—XI.)

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As stated in a slip inserted opposite the first portrait-plate of this series, the collection herein presented is issued without regard to chronological order, moreover, the portraits are confined to those men of science who, in various ways, have advanced the subject of parasitology.

The notes that follow are intentionally written in a brief form, their object being to point out the main contributions to parasitology made by those whose portraits are included in the series whilst giving references to sources whence further information may be gathered by those requiring it.

The collection of portraits that I have formed in the course of years and placed upon the walls of my laboratory, has frequently aroused the interest of my colleagues. Latterly the collection has been augmented, and it seems desirable to publish some of the portraits it contains, thereby rendering them available to a wider circle.

Antony van Leeuwenhoek

1632–1723.

(Portrait-plate I, facing p. 1.)

LEEUWENHOEK was born in 1632 and died, aged 91 years, on 26 August 1723¹ at Delft, Holland, where he lies buried in the Oude Kerk. He spent his early years as a linen draper, but when 22 years old obtained a sinecure office in his native town whereby he secured ample leisure for the pursuit of his scientific tastes. He learnt the art of lens grinding and is stated to have constructed some 250 microscopes, many of the instruments being specially adapted for particular investigations. With these instruments he examined everything that aroused his interest. In 1673 R. de Graaf sent Leeuwenhoek's first communication to the Royal Society, and subsequently Leeuwenhoek addressed many letters to the Society some of which appeared in the *Proceedings*, whilst

¹ On Plate I read 1723 for 1725.

others are preserved among the MSS. in the Library of the Society. In 1680 he was elected a Foreign Member of the Royal Society.

He made numerous observations of broad biological interest, amongst many things discovering the spermatozoa and demonstrating the capillary circulation. He made important contributions to parasitology. He described free living Protozoa (1676) and was the first to discover parasitic Protozoa, for, in a letter dated 4 Nov. 1681, to Robert Hooke, Secretary of the Royal Society, he gave a brief but unmistakable description of *Giardia* (*Lamblia*) *intestinalis* which he obtained from his own stools when loose after errors in diet(7). In his letter of Sept. 1683, and 16 Oct. 1692, he described the discovery of minute motile organisms (*Bacteria*) in the "materia alba" from between his teeth. Not only was he the first to discover bacteria but he was the first to figure them(1, 3). He appears to have been the first to raise *Pediculus humanus* experimentally, doing so upon his own person. He also studied the mechanism of feeding(4, 6) and the structure of the mouthparts of the louse, and in 1697 he described and figured nits(2). Of his work on *Pulex irritans* my late friend Professor H. G. Plimmer, F.R.S.(5) wrote:

"He glorified even in the common flea—so carefully collected for him by his little maidservant—and made the first exact observations upon this enemy of man. He described its structure and traced out the whole history of the metamorphoses of 'this minute and despised creature,' which some asserted to have been produced from sand, dust, dung of pigeons, or urine; and he showed that it was 'endowed with as great perfection in its kind as any large animal.' He also found that its pupa was attacked by a mite, the knowledge of which fact gave rise later to the well-known lines of Swift (1667–1745):

So, naturalists observe, a flea
Has smaller fleas that on him prey;
And these have smaller still to bite 'em,
And so proceed *ad infinitum*."

The two portraits reproduced in our plate were photographed by me in Göttingen in 1893 from the frontispieces to the 1695 and 1719 editions of Leeuwenhoek's works. The first shows him at the age of 63 (1695), the legend on the frontispiece reading "Antonius a Leeuwenhoek Regiae Societatis Londinensis Membrum....J. Verkolje pinx. A. de Blois fec." The second portrait (1719) shows him in his old age, the head being in an oval bearing an inscription and held above the clouds by a trumpeting angel of fame; this frontispiece bears various allegorical figures, statues, and a distant view of Delft in the background. His monument at Delft, which I visited in 1893, bears a medallion in marble; the head is shown in profile, the forehead being traversed by very deep wrinkles which long years of work with microscopes must surely have helped to chisel!

References: (1) Leeuwenhoek (1695), *Arcana Natura Detecta*, Delphis Batavorum, pp. 41–46, 53, pl. opposite 192, 187, 335–338 and pl. (2) Leeuwenhoek (1697), *Brieven*, p. 204 and pl. (3) Leeuwenhoek (1719), *Epistolae physiologicae super compluribus naturae arcanis*, etc.

Delphis. (4) Leeuwenhoek (1792), *Opera omnia seu Arcana Naturae*, Leyden; also translation by Hoole (1798–1800). (5) Plimmer (1913), the President's address: "Bedellus immortalis," *Journ. Roy. Microsc. Soc.* pp. 121–135. (6) Nuttall (1917), *Parasitology*, x. 104, 171 (gives citations). (7) Dobell (1920), The discovery of the intestinal Protozoa of man. *Proc. Roy. Soc. Med.* XIII. 1–15. Biographical notes on Leeuwenhoek will be found in most of the dictionaries.

Francesco Redi

1626–1697.

(Portrait-plate II, facing p. 96.)

REDI was born 18 February, 1626, at Arezzo in Tuscany. He was of noble family. He took the degrees of M.D. and Ph.D. at Pisa and became chief physician to Grand Duke Ferdinand II and later to Cosimo III in Florence. A man of broad culture, he took a leading position in science and letters; his genius being universally recognised. A collection of sixty of his poems was published by Prince Ferdinand of Tuscany in 1702. During the last nine years of his life he suffered much from epilepsy, his death occurring suddenly in the night of February 28th to March 1st, 1697, when he was with the Court at Pisa. He was accorded a public funeral and was buried in the church of San Francesco at Arezzo where his nephew, the bailiff Gregorio Redi, erected a handsome monument to him bearing the brief inscription: "Francesco Redi Patricio Aretino Gregorius Fratris Filius."

We cannot enter into Redi's activities as a physician. It should be noted, however, that he was the first to experiment with snake venom, finding that the venom is innocuous when taken by the mouth, and most toxic when it enters the circulation.

As a naturalist he was best known through his admirable researches upon the generation of insects and his observations on parasites. He (1668) exploded the old and widespread belief in the spontaneous generation of maggots in meat by protecting meat from blowflies by means of gauze and thereby laid the foundation for the belief *omne vivum ex vivo*. Parasites had been also supposed to be spontaneously generated and therefore Redi next turned his attention to these and found that sexual generation was not confined to higher animals as commonly believed. He found intestinal worms in which the two sexes occurred, that they laid eggs and he described (1684), amongst others, the reproductive organs of *Ascaris* of man. His treatise deals with the helminthology of various animals, not merely with worms occurring in the gut but likewise with worms living in the kidneys of mammals, air sacs of birds and the natatory sac of fish. He studied the parasites of the lion, bear, seal, eagle, mole, etc. and extended his investigations to Invertebrata (Cephalopods and Crustacea). Redi is therefore one of the fathers of parasitology, he was the first to make consecutive observations on Entozoa of animals and his researches were the first to stimulate scientific men to devote their attention to parasitic worms. F. de Filippi (1837), when he discovered the larva of *Fasciola hepatica* in *Planorbis nitidus* named it *Redia* in honour of Redi, who

first described the liver fluke. In his work of 1668 he lists and figures *Pediculi* and *Acari* parasitic on birds and mammals. Guiart (1898, v. *infra*) has determined many of the helminths studied by Redi and from the list he (G.) gives it is clear that in his pioneer treatise on parasitology Redi includes the principal types of each group. He also turned his attention to *scabies* in which Bonome and Cestoni of Livorno discovered *Sarcoptes* to be the cause. Redi recommended red mercurial ointment for the treatment of scabies, the application to be continued for some days after apparent recovery. Guiart lists 58 species of determinable Cestodes, Trematodes and Nematodes, 3 sp. of Acanthocephali, several species of Acari (ticks, itch-mites, etc.) and Insects (*Hypoderma*, *Oestrus*, *Cephenomyia*, and many species of *Pediculidae*) described and figured by Redi.

His most important parasitological publication (1684) is entitled "Osservazioni intorno agli animali viventi, che si trovano negli animali viventi" Firenze. See also: (1687) "Osservazioni intorno a' Pellicelli del corpo umano" Firenze, containing a letter by Giovancosimo Bonome to Redi, revised and added to by Redi prior to its publication; this deals with scabies. (1668) "Esperienze intorno alla Generazione degl' Insetti" Firenze.

An admirable biography of Redi by Guiart will be found in *Arch. de Parasitologie*, 1898, i. 420-441, with portrait and figures of the portrait medal (1684), etc. There were four medals struck in Redi's honour in 1677, 1684 and 1685, whilst his portrait was executed by van Kaathoven. A portrait was hung in the City Hall of Arezzo in 1699. The *Index Catalogue of the Surgeon General's Library*, Washington, 1st ser., xi. 1090, gives the following biographical references: *Gazz. Med. di Milano*, 1847, vi. 213, 265; *Gazz. Med. publ. Napoli*, 1875, vi. 161-174.

Carolus Linnaeus

1707-1778.

(Portrait-plate III, following II.)

LINNAEUS was born 23 May, 1707 (n.s.), at Râshult, Sweden, and died 10 January, 1778, at Upsala, where he lies buried in the Cathedral. He studied at Lund and at Upsala, and in 1741 became professor of medicine at Upsala, but soon after changed over to the chair of botany. His interests became centred in botany at an early age. His famous *Systema Naturae* appeared in 1735, the 10th edition of the *Systema Naturae* (Regnum Animale), which appeared in 1758, represents the foundation stone of modern zoological nomenclature. As the consecutive editions of this work appeared, they contained lists of scientific names that grew in length. In his *Oländska och Gothländska Resa* (1745) the index shows the first employment of specific names, thus ushering in the binomial nomenclature which we use to-day. He systematised the three kingdoms of nature and even drew up a treatise on *Genera Morborum*. A great many parasitic helminths first appear under their scientific

names in Linnaeus's edition of 1788. Apart from his having brought order out of chaos, Linnaeus evolved a method of giving terse descriptions which recorded the salient characters of animals and plants and greatly facilitated the differentiation of species. His lectures at Upsala attracted many students from different parts of the world. In 1753 he was created Knight of the Polar Star (figured in our portrait), this being the first time that a scientific man had been so honoured. He was raised to the nobility in 1761 and thereafter styled himself Baron Carl von Linné.

Biographical notices of Linnaeus will be found in most of the dictionaries and encyclopedias. For bibliography see Stiles and Hassall's *Index Catal. of Med. and Veter. Zool.*, Washington.

Carl de Geer

1720–1778.

(Portrait-plate IV, follows III.)

DE GEER was born 1720 and died 8 March, 1778, at Stockholm, Sweden. Of Dutch extraction, he was destined for a political life, but he turned to science. Possessed of great wealth, he consecrated a part thereof to good works—by helping, in the public interest, to repair inundated mines that put many miners out of employment. He became Court Marshal to the King of Sweden and Commander of the Order of Vasa. He made extensive collections which he left to the Stockholm Academy of Sciences.

His main work, *Mém. pour servir à l'hist. des Insectes*, in 8 vols., 4°, appeared in 1752–1778 in Stockholm. To parasitologists his name will be familiar in connection with Acari and parasitic insects of which he founded many species.

A good biography will be found in *Biographie universelle ancienne et moderne*, rédigée par une société de gens des lettres, etc., Paris, 1816, vol. XII. p. 19. More details are obtainable in *Svenska Vetenskapsakademiens Handlingar* 1779, which contains the memorial speech by Bergmann (in Swedish). These references were kindly given to me by Dr E. Nordenskiöld (Stockholm), who helped me to obtain the photograph we reproduce of de Geer's portrait, which hangs in Stockholm Academy of Sciences.

Karl Asmund Rudolphi

1771–1832.

(Portrait-plate V, facing p. 192.)

RUDOLPHI was born 14 July, 1771, at Stockholm, Sweden. In 1790 he went to Greifswald to pursue his studies in medicine and natural science. He took the degrees of Ph.D. (1793) and M.D. (1795) at Greifswald, both of his dissertations for these degrees relating to Entozoa. He became Privatdocent in the Philosophical (1793) and Medical (1795) Faculties at Greifswald, and

practised medicine for a brief period only. In 1808 he became full Professor of Medicine at Greifswald and in 1810 he went to Berlin as Professor of Anatomy and Physiology. He died in Berlin, 29 November, 1832.

Rudolphi acquired a remarkably broad training in natural science. His publications related to Botany, Zoology, Anatomy, Pathology, Physiology, Mineralogy, Veterinary Science and Medicine. In Berlin he made extensive additions to the collections of comparative anatomy, etc. His helminthological collections are preserved at the Berlin Zoological Museum. He was a most distinguished teacher and a man of stimulating character.

Rudolphi's work in helminthology was of fundamental importance and stands out as his most valuable contribution to science. His chief publication, entitled *Entozoarum historia naturalis* (1808–1810) in 3 vols., has served for helminthology in a manner that may be compared to that of Linnaeus's *Systema Naturae* in its bearing on systematic zoology. Rudolphi was the first to put helminthology upon a scientific basis. His *Synopsis entozoorum* appeared in 1819.

For a full biography see Lühe (1900): "Karl Asmund Rudolphi der Vater der Helminthologie," *Arch. de Parasitologie*, III. 549–577, with portrait and facsimile of his writing. For a bibliography of Rudolphi's helminthological papers see Stiles and Hassall's *Index*. The portrait we reproduce is from a contemporary engraving.

Johannes Müller

1801–1858.

(Portrait-plate VI, follows V.)

BORN 14 July, 1801, at Coblenz, Johannes Müller, the son of a shoemaker, died 28 April, 1858, in Berlin, as Professor, and was recognised throughout the world of science as one of the greatest of biologists. He studied theology at Bonn (1819) and afterwards medicine, winning a prize for his first essay entitled *Respiration of the foetus* (1823), after which he went to Berlin to pass his examination and there fell under the influence of Rudolphi. He became Privatdocent at Bonn (1824), and, on Rudolphi's death, he went to Berlin (1833) as Director of the Anatomical School and Museum. He taught anatomy (human and comparative), pathology and physiology. Fundamental were his researches on glands. With Purkinje, he was the first to apply the microscope to the study of animal tissues and, helped by his pupils, he laid the foundations of modern histology. Müller was the first to recognise "connective tissue."

In the course of 25 years Müller published no less than 200 papers. His contributions to parasitology relate to *Hirudo vulgaris*, *Ixodes ophiophilus* Müller; he discovered the *Myxosporidia* and *Entoconcha mirabilis*, a remarkable parasitic snail occurring in the body-cavity of *Synapta digitata*, a Holothurian.

For biography see Lühe (1902), *Arch. de Parasitologie*, v. 95–117; references to publications will be found in Stiles and Hassall's *Index*. A bronze

statue of Johannes Müller stands before the Stadthaus at Coblenz. The well-known portrait of Müller which we reproduce was taken in 1857, a year before his death. The face shows extraordinary energy.

Joseph Leidy

1823–1891.

(Portrait-plate VII, follows VI.)

LEIDY was born 9 September, 1823, and died 30 April, 1891, at Philadelphia. He was of French-German extraction, his forefather Carl Leidy having migrated to Pennsylvania about 1704. At an early age Leidy showed an aptitude for drawing, a talent that he made use of in connection with his scientific work. After taking his M.D. at the University of Pennsylvania in 1844, he practised medicine for two years. He then took up natural history and became Prosector and Curator of the Academy of Sciences of Philadelphia (1846) at the age of 23. When he was 30 he became Professor of Anatomy, subsequently he was Professor of Zoology and Comparative Anatomy.

Of Leidy's 550 papers, no less than 120 relate to helminths. His contributions cover a wide field however, for they relate to mineralogy, botany, vertebrate and invertebrate comparative anatomy, and palaeontology. His papers on Protozoa and Helminths were important. He discovered *Trichina spiralis* in the pig (1846), and this led Leuckart to make the important suggestion that man might become infected by eating raw pork. In 1850–1856 he wrote on various helminths and his *Synopsis of Entozoa* appeared in 1856. In 1871 he commented on flies as agents in the carriage of contagious disease, and from this time onwards he seems to have worked mainly on parasites and Rhizopods. He wrote on miscellaneous parasites, Gregarines, Cestodes, Trematodes, Nematodes, Hirudinea, parasitic Crustacea, Acari and Insecta. All who knew him remember him as possessed of a most attractive personality.

For biography see H. B. Ward (1900), *Arch. de Parasitologie*, III. 269–279, with portrait as an older man, facsimile signature and letter and a short list of his parasitological papers. A fuller bibliography will be found in Stiles and Hassall's *Index* (1906, pp. 1050–1057) and in Leidy's *Researches in Helminthology and Parasitology*, published by Joseph Leidy jr. in 1904. Leidy left much work on helminths and gregarines unpublished. I am indebted to his sons for the excellent portrait herein reproduced.

Edward Jenner

1749–1823.

(Portrait-plate VIII, facing p. 404.)

THE discoverer of vaccination, EDWARD JENNER was born on 17 May, 1749, and died on 26 January, 1823, at Berkeley, Gloucestershire, where he lies buried in the Parish Church. The son of a clergyman, he took an interest in natural



EDWARD JENNER

1749—1823

From the picture by James Northcote, R.A., in the National Portrait Gallery
Photographed by Messrs Emery Walker, Ltd., London

history from childhood. In 1770 he became a resident pupil for two years in the house of John Hunter who greatly influenced him. He studied at St George's Hospital and began to practise medicine in 1773 at Berkeley. He published some papers on natural history, became F.R.S. in 1788 and M.D. (St Andrew's) in 1792.

Owing to a local belief, known to him from childhood, that dairy-maids escape smallpox, he sought to test the belief by experiment. On 14 May, 1796, he vaccinated a boy of eight with cowpox, produced cowpox, and on 1 July he inoculated the boy with smallpox with negative result. In June, 1798, he published his first account of his observations and conclusions: *An Inquiry into the Cause and Effect of Variolae Vaccinae*, etc. In 1802 he received a grant of £10,000 and in 1806 one of £20,000 for his discovery. Oxford conferred the degree of M.D. (hon. causa) upon him in 1813. Dibdin, in his *Reminiscences*, says: "I never knew a man of a simpler mind or of a warmer heart than Dr Jenner."

For biography see John Baron (1838), *Life of Edward Jenner*, 2 vols., also *Dict. National Biogr.* xxix. 321–324. Portraits: (1) by Sir Thos. Lawrence, (2) by James Northcote, R.A. (in the National Portrait Gallery), (3) marble statue in Gloucester Cathedral, (4) bronze statue in Kensington Gardens, etc.

Richard Owen

1804–1892.

(Portrait-plate IX.)

RICHARD OWEN, distinguished as a biologist, comparative anatomist and palaeontologist, was born 20 July, 1804, at Lancaster, and died at Sheen Lodge, Richmond Park, on 18 December, 1892; he lies buried at Ham in Surrey. He studied medicine at Edinburgh and St Bartholomew's, and became Curator of the Museum of the Royal College of Surgeons, London. In 1834–1835 he was Professor of Comparative Anatomy, lecturing at St Bartholomew's and at the Royal College of Surgeons. In 1856 he became Superintendent of the Natural History Department of the British Museum, but continued teaching. A Fellow of the Royal Society, he was created K.C.B. in 1884.

He published about 400 contributions to science, these concerning "almost every class of animal from sponge to man"; his essay on Parthenogenesis (1849) represented pioneer work. His parasitological papers (see Stiles and Hassall's *Index of Vet. and Med. Zool.*, 1908, pp. 1620–1623) deal with *Trichina spiralis*, which he named in 1835, *Linguatula*, *Distoma*, *Taenia*, *Gnathostoma*, etc.

For his biography see Rev. Richard Owen (1894), *The Life of Richard Owen by his grandson...including an essay by Huxley*, London, 8°, 409 pp., figs. and pls. Of his many portraits we reproduce the upper part of that by H. G. Pickersgill, R.A., which was presented in 1893 by Owen's daughter-in-law to the National Portrait Gallery in accordance with his wish.

Athanasius Kircher

1601–1680.

(Portrait-plate X.)

A CLASSICAL scholar, Egyptologist, astrologist, mathematician and natural philosopher, ATHANASIUS KIRCHER was born at Geisa, near Fulda, on 2 May, 1601. He died as a teacher of mathematics in Rome on 30 October, 1680. He was a Jesuit, and held a professorship at Würzburg (1631), where he published his *Ars Magnesia* dealing with "Magnetismus." Ultimately through the influence of Cardinal Berberini, he went as a teacher to the Collegium Romanum, Rome, where he founded the collection known to this day as the "Museum Kircherianum."

Biologists will associate his name with the "experimentum mirabile," i.e. the hypnotisation of the fowl, which he illustrates in his *Physiol. Kircheriana* (1680), the fowl lying prone with its outstretched head on a line drawn on flagstones. Moreover, his writings had great influence in establishing the doctrine of "contagium animatum." In his *Scrutinium Physico-Medicum contagiosae luis quae dicitur Pestis*, etc. (Lipsiae, 1671), whose Introduction is dated 22 February, 1658, by Kircher, he states (p. 25) that he found countless numbers of minute worms in putrefying substances. Upon this he based various conclusions regarding the etiology of the plague which raged in Italy in 1656, for, with his simple microscope, he reported having discovered uncountable numbers of minute worms in the blood and bubo-pus of plague patients. No doubt Kircher merely saw the blood corpuscles and leucocytes or pus cells and called these "worms," but his teaching carried great weight and doubtless gave rise to the view subsequently expressed by Robert Boyle (*vide infra*). Our portrait is taken from Kircher's *Mundus Subterraneus* (1665). Further particulars regarding his publications and biography will be found in the *Index Catalogue of the Surgeon-General's Library*, Washington.

Robert Boyle

1627–1691.

(Portrait-plate XI.)

NATURAL philosopher, chemist, and versatile genius, the Hon. ROBERT BOYLE was born at Lismore Castle, Waterford, on 25 January, 1627, as the seventh son and fourteenth child of the first Earl of Cork. He died 30 December, 1691, in London, where he lies buried in St Martin's-in-the-Fields. References to his contributions to science, etc., will be found in the *Dictionary of National Biography*, VI. 118–123. His complete works, with his correspondence and a *Life* by Dr Birch were published (1744) in 5 vols. He took a leading part in founding the Royal Society.

To biologists he will be remembered for his fundamental discoveries on the physiology of respiration, but in addition he wrote with prophetic insight

regarding the causation of infective diseases. In his essay on the Pathological Part of Physic he wrote "and let me add that he that thoroughly understands the nature of ferments and fermentation shall probably be much better able than he that ignores them to give a fair account of diverse phenomena of several diseases (as well as fevers and others) which will perhaps be never properly understood without an insight into the doctrine of fermentation." In these words, written some 240 years ago, we trace the beginnings of what became the "germ theory" of disease.

Our portrait is taken from the painting by Frederic Kerseboom in the collection of the Royal Society, London.

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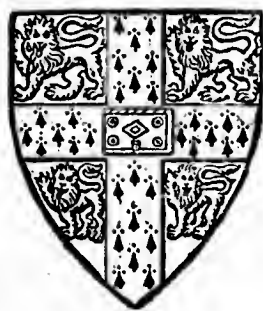
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EDITED BY

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